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(54) Title: REGULATION OF INSULIN PRODUCTION

(57) Abstract: Gene therapy-based treatment of type-2 diabetes, obesity, and related conditions by *in vivo* expression of glucagon-like peptide 1 (GLP-1), and/or glucose-dependent insulinotropic peptide (GIP). The treatment may be combined with concurrent administraton of dipeptidyl peptidase IV (DPP-IV) inhibitors.

REGULATION OF INSULIN PRODUCTION

This application claims the benefit under 35 USC §119(e) of United States Provisional Patent Application No. 60/328,116, filed October 9, 2001, for "Regulation of Insulin Production in a Mammal." The disclosure of this application is incorporated herein by reference in its entirety.

Background of the Invention

The invention provides gene therapy-based treatment of type-2 diabetes, obesity, and related conditions by in vivo expression of glucagon-like peptide 1 (GLP-1), and/or glucose-dependent insulinotropic peptide (GIP). The treatment may be combined with concurrent administration of dipeptidyl peptidase IV (DPP-IV) inhibitors.

Type-2 diabetes is characterized by hyperglycemia, hyperinsulinemia, and hyperlipidemia. Impaired insulin release from pancreatic β-cells and insulin resistance in peripheral tissues (skeletal muscle and adipose tissues) results in impaired peripheral glucose disposal and causes hyperglycemia. Insulin resistance causes a compensatory increase in insulin secretion from pancreatic β-cells, which results in hyperinsulinemia. Increased fatty acid flux to the liver, caused by insulin resistance, increases lipoprotein production and results in hyperlipidemia. Therefore, a mechanism to allow glucose-induced insulin secretion would provide an improved therapy for type 2 diabetes.

GLP-1 is a peptide synthesized in intestinal L cells by proteolytic cleavage of the preproglucagon molecule. Functions of GLP-1 include the enhancement of regulated secretion of insulin from pancreatic β-cells in response to increased blood glucose levels and suppression of glucagon secretion, which together results in a decrease in blood glucose levels without causing hypoglycemia (Thorens, 1995; Kieffer and Habener, 2000). Other functions of GLP-1 include delayed gastric emptying and a reduction in appetite and food intake (Gutzwiller et al., 1999; Flint et al., 1998; 2000).

GLP-1 has an extremely short half-life in vivo (<2 minutes). In man, GLP-1 is quickly inactivated by DPP-IV. Therefore, much effort has been placed on the development of GLP-1 analogues resistant to DPP-IV degradation (Burcelin et al., 1999; Gallwitz et al., 2000), as well as natural GLP-1 analogues isolated from other species such as exendin 4 (Hughes, 1998; Greig et al., 1999; Young et al., 1999). Alternatively, others have focused on

the development of small molecule inhibitors of DPP-IV, which would inhibit GLP-1 degradation and functionally increase the half-life of GLP-1 (Holst and Deacon, 1998).

Infusion of GLP-1 peptide into diabetic patients results in normalization of blood glucose levels (Nauck et al., 1993; 1998; Habener, et al. 1993; Rachman et al., 1997) and GLP-1 administration has been demonstrated to reduce food intake in humans (Flint et al., 1998; Gutzwiller et al., 1999). Therefore, it may play a role in controlling satiety. These data suggest that GLP-1 is a potentially therapeutic agent for use in improving β-cell function and glycemic control in type 2 diabetes.

GIP, a peptide synthesized by duodenum K cells, functions to stimulate insulin release in response to increased blood glucose levels and may also have the advantage of lowering blood lipid levels (Kieffer and Habener, 2000). Unlike GLP-1, GIP has not been demonstrated to improve the phenotype of diabetic patients (Service et al., 1990), although GIP has been shown to enhance insulin-mediated glucose disposal in sheep (Rose et al., 1988), rats (O'Harte et al., 1999) and mice (O'Harte et al., 2000). Therefore, the expression of GIP in combination with GLP-1 may provide an improved therapy that surpasses the effect of each peptide alone. Finally, a recent study has demonstrated that DPP-IV inhibition reduces GIP degradation and potentiates its insulinotropinic and antihyperglycemic effects in pigs (Deacon et al., 2001).

A significant limitation to potential GLP-1 and GIP therapy in diabetic patients is the short biological half-life that makes protein replacement therapy problematic. In addition, the endogenous expression of GLP-1 and GIP, combined with the use of a small molecule DPP-IV inhibitor, will increase the circulating levels of both hormones and add a means to control GLP-1 and GIP steady-state levels. The insulinotropic hormones, GLP-1 and GIP, are metabolized rapidly by the ubiquitous enzyme, DPP-IV, resulting in the formation of an inactive, or, in some cases, an antagonistic peptide. DPP-IV has a dual function as a regulatory protease and as a binding protein. DPP-IV cleaves Xaa-Pro or Xaa-Ala dipeptides from the N-terminus of regulatory peptides. This minimal truncation of peptides by DPP-IV results in inactivation of many mammalian regulatory peptides including: neuropeptides such as neuropeptide Y or endomorphin, circulating peptide hormones like peptide YY, growth hormone-releasing hormone, GLP-1, and GLP-2, GIP, and many others (reviewed by Mentelin, 1999). Due to this rapid degradation, the effects of single, bolus protein administration of GLP-1 and/or GIP peptide are short lasting, and for demonstration of the

peptide antidiabetogenic effects, continuous intravenous infusion is required. Therefore, constant level, endogenous secretion of these incretin hormones may provide an advantage over protein replacement therapy. This constant level endogenous secretion can be achieved by in vivo expression of GLP-1 and/or GIP-1.

Gutless adenoviral vectors have been shown to achieve high-level transgene expression and improved duration of expression with reduced vector toxicity. Such vectors also have a large coding capacity (up to 38 kb) and can encode multiple transgene expression cassettes including ligand-regulated transcriptional control elements (Kochanek, 1999).

Retroviral vectors and lentiviral viral vectors have a coding capacity of approximately 8 kb, and the viral genome is capable of integration into the host cell chromosome, thus allowing for potentially life-long transgene expression. One non-limiting example of a lentivirus is Bovine-Immunodeficiency-Virus (BIV). (WO 01/44458).

AAV vectors integrate into the host genome and such vectors are capable of long-term transgene expression. The coding capacity of AAV vectors is approximately 5 kb.

Summary of the Invention

In view of the above, the present invention provides the following exemplary enumerated embodiments:

1. A viral vector comprising:

(a) a polynucleotide sequence encoding GLP1;

(b) a polynucleotide sequence encoding a signal sequence upstream of (a); and

(c) a polyadenylation signal downstream of (a).

2. The vector according to embodiment 1, further comprising a polynucleotide sequence encoding a proteolytic cleavage site located between said polynucleotide sequence encoding GLP1 and said polynucleotide sequence encoding the signal sequence.

3. The vector according to embodiment 1 or 2, wherein GLP1 comprises SEQ ID NO:2, 4, 6, 8, or 10.

4. The vector according to embodiment 1 or 2, wherein said polynucleotide sequence encoding GLP1 comprises SEQ ID NO:1, 3, 5, 7 or 9.

5. The vector according to any of embodiments 1-4, wherein said signal sequence is an IgK signal sequence.

6. The vector according to embodiment 5, wherein said IgK signal sequence comprises SEQ ID NO:18.

7. The vector according to embodiment 6, wherein said polynucleotide encoding the IgK signal sequence comprises SEQ ID NO:17.

8. The vector according to any of embodiments 1-4, wherein said signal sequence is an exendin signal sequence.

9. The vector according to embodiment 8 wherein said exendin signal sequence comprises SEQ ID NO:16.

10. The vector according to embodiment 9, wherein said polynucleotide encoding the exendin signal sequence comprises SEQ ID NO:15.

11. The vector according to any one of the preceding embodiments, wherein the polyadenylation signal is derived from SV40.

12. The vector according to any one of embodiments 2-11, wherein said proteolytic cleavage site is cleaved by furin protease.

13. The vector according to any one of the preceding embodiments, wherein expression of said polynucleotide sequence encoding GLP1 is controlled by a regulatable promoter.

14. The vector according to any of embodiments 1-13, wherein said vector is an adenoviral vector.

15. The vector according to any of embodiments 1-13, wherein said vector is a retroviral vector.

16. The vector according to any of embodiments 1-13, wherein said vector is a lentiviral vector.

17. The vector according to any of embodiments 1-13, wherein said vector is an adeno associated viral vector.

18. The vector according to any one of the preceding embodiments, further comprising a polynucleotide encoding GIP.

19. The vector according to embodiment 18, wherein GIP comprises SEQ ID NO:14.

20. The vector according to embodiment 19, wherein said polynucleotide sequence encoding GIP comprises SEQ ID NO:13.

21. A mammalian cell comprising the vector according to any one of embodiments 1-20.

22. A packaging cell comprising the vector according to any one of embodiments 1-20.

23. A method of making a viral particle comprising culturing the packaging

cell of embodiment 22 under conditions wherein the particle is produced.

24. A method of treating diabetes in a mammal comprising administering a physiologically effective amount of the vector in any one of embodiments 1-20 to the mammal.

25. The method of embodiment 24, wherein the mammal is a primate.

26. The method of embodiment 25, wherein the primate is a human.

27. The method of treating diabetes according to any one of embodiments 24-26, further comprising administering a DPP-IV inhibitor to said mammal.

28. A viral vector comprising:

(a) a polynucleotide sequence encoding GIP;

(b) a polynucleotide sequence encoding a signal sequence upstream of (a); and

(c) a polyadenylation signal downstream of (a).

29. The vector according to embodiment 27, further comprising a polynucleotide sequence encoding a proteolytic cleavage site located between said polynucleotide sequence encoding GIP and said polynucleotide sequence encoding the signal sequence.

30. The vector according to embodiment 28 or 29, wherein GIP comprises SEQ ID NO:14.

31. The vector according to embodiment 30, wherein said polynucleotide sequence encoding GIP comprises SEQ ID NO:13.

32. The vector according to any of embodiments 28-31, wherein said signal sequence is an IgK signal sequence.

33. The vector according to embodiment 32, wherein said IgK signal sequence comprises SEQ ID NO:18.

34. The vector according to embodiment 33, wherein said polynucleotide encoding the IgK signal sequence comprises SEQ ID NO:17.

35. The vector according to any of embodiments 28-31, wherein said signal sequence is an exendin signal sequence.

36. The vector according to embodiment 35, wherein said exendin signal sequence comprises SEQ ID NO:16.

37. The vector according to embodiment 36, wherein said polynucleotide encoding the exendin signal sequence comprises SEQ ID NO:15.

38. The vector according to any one of embodiments 28-37, wherein the polyadenylation signal is derived from SV40.

39. The vector according to any one of embodiments 29-38, wherein said proteolytic cleavage
site is cleaved by furin protease.

40. The vector according to any one of embodiments 28-39, wherein expression of said polynucleotide sequence encoding GIP is controlled by a regulatable promoter.

41. The vector according to any of embodiments 28-39, wherein said vector is an adenoviral vector.

42. The vector according to any of embodiments 28-39, wherein said vector is a retroviral vector.

43. The vector according to any of embodiments 28-39, wherein said vector is a lentiviral vector.

44. The vector according to any of embodiments 28-39, wherein said vector is an adeno associated viral vector.

45. The vector according to any one of embodiments 28-44, further comprising a polynucleotide sequence encoding GLP1.

46. A mammalian cell comprising the vector according to any one of embodiments 28-45.

47. A packaging cell comprising the vector according to any one of embodiments 28-45.

48. A method of making a viral particle, comprising culturing the packaging
cell according to embodiment 47 under conditions wherein the particle is produced.

49. A method of treating diabetes in a mammal, comprising administering a physiologically effective amount of the vector according to any one of embodiments 28-45 to the mammal.

50. The method of embodiment 49, wherein the mammal is a primate.

51. The method of embodiment 50, wherein the primate is a human.

52. The method of treatment as specified in any one of embodiment 49-51, further comprising
administering a DPP-IV inhibitor to said mammal.

Brief Description of the Drawings

Figure 1A Map of plasmid pCiGLP1IgK+Ala

Figure 1B Map of plasmid pCiGLP1Ext

Figure 1C Map of plasmid pCi-nGLP1IgkplfHis

Figure 2 Adenoviral vectors encoding Tet system

Figure 3A Map of plasmid pGTL14GLP+Ala

Figure 3B Map of plasmid pGTL15GLP+Ala

Figure 3C Map of plasmid pGTL146GLPex

Figure 3D Map of plasmid pGTL15GLPex

Figure 3E Map of plasmid pGTI.14GLPIgKplf

Figure 3F Map of plasmid pGTI.15GLPIgKplf

Figure 4A Map of plasmid pBV2.CGE.C7.GLP-1Ex

Figure 4B Map of plasmid pAGVC7endo1c

Figure 4C Map of plasmid pGTL24aPL2

Figure 4D Map of plasmid pGTIAPL2.CGE.C7.GLP-1Ex

Figure 4E Map of plasmid pbv2

Figure 5A Map of plasmid pBV2.CMV.GLP-1Ex

Figure 5B Map of plasmid pGTLAPL2.CMV.GLP-1Ex

Figure 6A Map of plasmid pGTI.APL2.ALB.GLP-1Ex

Figure 6B Map of plasmid pBV2.ALB.GLP-1Ex

Detailed Description of the Invention

The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics, immunology, cell biology, cell culture and transgenic biology, which are within the skill of the art. See, e.g., Maniatis et al., 1982; Sambrook et al., 1989; Sambrook and Russell, 2001; Ausubel et al., 1992; Glover, 1985; Anand, 1992; Guthrie and Fink, 1991; Harlow and Lane, 1988; Jakoby and Pastan, 1979; Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); Transcription And Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Gene Transfer Vectors For

Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Methods In Enzymology, Vols. 154 and 155 (Wu et al. eds.), Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); Riott, Essential Immunology, 6th Edition, Blackwell Scientific Publications, Oxford, 1988; Hogan et al., Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

As used in this specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, a virion particle includes a plurality of virion particles.

In one embodiment of the invention, a nucleic acid or multiple nucleic acids encoding GLP-1 and/or GIP-1 are delivered to a cell, causing the cell to express increased amounts of GLP-1 and/or GIP-1. The cells of this embodiment may either be cultured *in vivo* or *in vitro* and transduced with nucleic acids encoding GLP-1 and/or GIP-1. Preferably, the cell is eukaryotic; more preferably, mammalian; and most preferably, human. This cell of the invention when in vitro may be used to produce GLP-1 and/or GIP-1 protein. These protein(s) may be further purified for delivery to an animal or for further studies related to the effects of GLP-1 and/or GIP-1 on cells, including their regulating effects on insulin production. The purified proteins may be delivered to an animal to study their regulating effects on insulin production in the animal. Preferably, the animal is a mammal and most preferably, a human.

Preferably, nucleic acids encoding GLP-1 and/or GIP-1 are delivered to a cell via a gene delivery vector. Gene delivery vectors include, but are not limited to, plasmids, phage, cosmids or viral based vectors.

As used herein, the term "viral vector" is used according to its art-recognized meaning. The viral vectors of the invention may be utilized for the purpose of transfering DNA, RNA or other nucleic acids into cells either in vitro or in vivo. Viral vectors include, but are not limited to, retroviral vectors, vaccinia vectors, lentiviral vectors, herpes virus vectors (e.g., HSV), baculoviral vectors, cytomegalovirus (CMV) vectors, papillomavirus vectors, simian virus (SV40) vectors, Sindbis vectors, semliki forest virus vectors, adenoviral vectors, and adeno-associated viral (AAV) vectors.

One embodiment of the invention includes vectors encoding GLP-1 and/or GIP. The vectors can be used to deliver GLP-1 and/or GIP encoding sequences to cells in vitro or in vivo. Thus, these vectors are useful for expression of GLP-1 and/or GIP in vitro or in vivo. Another embodiment of the invention includes cells containing the vectors of the invention.

The invention also comprises polynucleotides that encode the proteins of the invention. As used herein, the term "polynucleotide" means a nucleic acid molecule, such as DNA or RNA, that encodes a polypeptide. The molecule may include regulatory sequences. Preferably, the polynucleotide is DNA. Such polynucleotides are prepared or obtained by techniques known by those skilled in the art in combination with the teachings contained therein. The polynucleotides of the invention also include polynucleotides that differ in certain bases but still encode the proteins of the invention due to the redundancy of the genetic code.

The genes of the invention can be optimized for enhanced expression in the organism of choice. In this manner, the genes can be synthesized utilizing preferred codons for the organism of choice. That is the most preferred codon for a particular host is the single codon, which most frequently encodes that amino acid in that host. For example, the human preferred codon for a particular amino acid may be derived from known gene sequences from humans. Synthetic genes can also be made based on the distribution of codons a particular host uses for a particular amino acid. It is understood that optimization of a gene does not require every codon encoding for a particular amino acid be changed to the most frequently used codon in that particular organism. Optimization requires at least one codon encoding for a particular amino acid be changed to a codon which is more frequently used in that organism for the same amino acid. It does not need to be the most frequently used codon. Preferably more than 5% of the codons are changed to an equivalent codon that is used more frequently in the particular organism. More preferably more than 10% of the codons are changed to an equivalent codon that is used more frequently in the particular organism. Most preferably more than 20% of the codons are changed to an equivalent codon that is used more frequently in the particular organism. By equivalent codon is meant that the codon encodes for the same amino acid in the organism.

In this manner, the nucleotide sequences can be optimized for expression in any organism. It is recognized that all or any part of the gene sequence may be optimized or synthetic. That is, synthetic or partially optimized sequences may also be used.

Recoding of a gene or portions of a gene can be performed using techniques well known in the art. By way of non-limiting examples, Casimiro DR et al. describes a PCR-based method for gene synthesis(Structure 1997 Nov 15;5(11):1407-12) (See also Brocca et al. "Design, total synthesis, and functional overexpression of the Candida rugosa lip1gene coding for a major industrial lipase" Protein Sci 1998 Jun;7(6):1415-22; Withers-Martinez C, et al., "PCR-based gene synthesis as an efficient approach for expression of the A+T-rich malaria genome" Protein Eng 1999 Dec;12(12):1113-20; and Stemmer et al., "Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides" Gene 1995 Oct 16;164(1):49-53).

A functional variant may differ in amino acid sequence by one or more substitutions, additions, deletions, truncations which may be present in any combination, but would retain the same biological function as the reference gene (see Biological function). The present invention includes vectors expressing a functional variant of GLP-1 and/or GIP.

"Biological function" within the meaning of this application is to be understood in a broad sense. It includes, but is not limited to, the particular functions of the GLP-1 and/or GIP protein disclosed in this application. Thus, biological functions are not only those which a polypeptide displays in its physiological context, i.e. as part of a living organism or cell, but includes functions which it may perform in a non-physiological setting, e.g. in vitro. For example, a biological function of the GLP-1 and/or GIP protein within the meaning of this application is the ability to, for example, demonstrate regulation of insulin production either *in vitro* or *in vivo*. Assays to assess the required properties are well-known in the art.

"Polynucleotide" and "nucleic acid", used interchangeably herein, generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single- stranded or more typically double stranded or a mixture of single- and double-stranded regions. In addition polynucleotide refers to triple- stranded regions comprising RNA or DNA or both RNA and DNA. "Polynucleotide" also includes a polymer comprising purine and pyrimidine bases, or other natural, chemically, biochemically modified, non-natural or derivatized nucleotide bases. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases

and DNAs or RNAs with backbones modified for stability or for other reasons. The backbone of the polynucleotide can comprise sugars and phosphate groups (as may typically be found in RNA or DNA), or modified or substituted sugar or phosphate groups. "Modifed bases" includes for examples tritylated bases and unusual bases such as inosine. A variety of modifications have been made to DNA and RNA; thus "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides. As used herein, "DNA" includes not only bases A, T, C, and G, but also includes any of their analogs or modified forms of these bases, such as methylated nucleotides, internucleotide modifications such as uncharged linkages and thioates, use of sugar analogs, and modified and/or alternative backbone structures, such as polyamides. The term "DNA" also includes cDNA, genomic DNA or synthetic DNA.

The terms "promoter", "promoter region", or "promoter sequence" refer generally to transcriptional regulatory regions of a gene, which may be found at the 5' or 3' side of the coding region, or within the coding region, or within introns. Typically, a promoter is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. The typical 5' promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence is a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

The invention includes the use of hybrid GLP-1 and/or GIP nucleotide sequences that contains a heterologous promoter, a heterologous signal sequence (to allow secretion), the coding region, and a poly(A) signal. This nucleotide sequence is encoded in a gene delivery vector. Preferable vectors include an adenovirus (preferably a "gutless" adenovirus), AAV, or lentiviral (preferably BIV) based vectors. Preferably, the promoter is a regulated promoter and transcription factor expression system, such as the published tetracycline-regulated systems, or other regulatable systems (WO 01/30843), to allow regulated expression of GLP-1 incorporated into gutless adenoviral or lentiviral vectors. Preferably, the vector is targeted to a specific cell type. Preferably the cell is a hepatocyte.

The term "adenoviral particle" is to be understood broadly as meaning infectious viral particles that are formed when an adenoviral vector of the invention is transduced into an appropriate cell or cell line for the generation of infectious particles. As used herein, the term "adenovirus" or "adenoviral particle" is used to include any and all viruses that may be categorized as an adenovirus, including any adenovirus that infects a human or an animal, including all groups, subgroups, and serotypes. Preferably, such adenoviruses are ones that infect human cells. Such adenoviruses may be wild-type or may be modified in various ways known in the art or as disclosed herein. Such modifications include modifications to the adenovirus genome that is packaged in the particle in order to make an infectious virus. Such modifications include deletions known in the art, such as deletions in one or more of the E1, E2a, E2b, E3, or E4 coding regions. Such modifications also include deletions of all of the coding regions of the adenoviral genome. Such adenoviruses are known as "gutless" adenoviruses. The terms also include replication-conditional adenoviruses; that is, viruses that replicate in certain types of cells or tissues but not in other types. These include the viruses disclosed in U.S. Patent No. 5,998,205, issued December 7, 1999 to Hallenbeck et al. and U.S. Patent No. 5,801,029, issued September 1, 1998 to McCormick, the disclosures of both of which are incorporated herein by reference in their entirety. Such viruses are sometimes referred to as cytolytic or cytopathic viruses (or vectors), and, if they have such an effect on neoplastic cells, are referred to as oncolytic viruses (or vectors).

In another embodiment, adenoviral vectors of the present invention are delivered by intravenous injection (IV) to an animal. Preferably the animal is a mammal; more preferably a primate and most preferably a human. These adenoviral vectors may be delivered at a dose of about $1x10^9$ to $5x10^{13}$ adenoviral vector particles per kilogram (kg); preferably at a dose of about $1x10^{10}$ to $1x10^{13}$ adenoviral vector particles per kg; and most preferably for replicating vectors (e.g. "oncolytic" vectors) about $5x10^{10}$ to $5x10^{11}$ and for replication defective vectors about $1x10^{10}$ to $1x10^{13}$ adenoviral vector particles per kg.

The adenoviral vectors of the invention are made by standard techniques known to those skilled in the art. The vectors are transferred into packaging cells by techniques known to those skilled in the art. Packaging cells provide complementing functions to the functions provided by the genes in the adenovirus genome that are to be packaged into the adenovirus particle. The production of such particles requires that the vector be replicated and that those proteins necessary for assembling an infectious virus be produced. The packaging cells are

cultured under conditions that permit the production of the desired viral vector particle. The particles are recovered by standard techniques. The preferred packaging cells are those that have been designed to limit homologous recombination that could lead to wild-type adenoviral particles. Such cells are disclosed in U.S. Patent Nos. 5,994,128, issued November 30, 1999 to Fallaux, et al., and 6,033,908, issued March 7, 2000 to Bout, et al. The packaging cell known as PER.C6, which is disclosed in these patents, is particularly preferred.

Lentiviral and retroviral vectors are generally constructed such that the majority of the viral genes are deleted and replaced by a gene of interest. Most frequently the gene of interest is transcribed under the control of the viral regulatory sequences within the long terminal repeat (LTR). Alternatively, the gene of interest may be expressed under the regulation of its own internal promoter. The genes which have been deleted from the vector are generally provided by one or more helper or packaging constructs in a packaging cell line (Bender et al., J. Virol. 61: 1639 – 1649 (1987) and Miller et al., Biotechniques, 7:980 –990 (1989)). Also see Markowitz et al., J. Virol. 62:1120 – 1124 (1988) wherein complementary portions of the helper construct were divided on two separate constructs. The packaging cell line may be transfected with the vector, thereby producing vector RNA that is packaged into the virus particles. These released virus particles are replication defective and can be used to deliver the vector carrying the gene of interest to target cells. Preferably, the vector is derived from a lentivirus and preferably, from BIV.

To increase safety, efficiency and accuracy of the recombinant vector systems, various improved recombinant systems have been constructed. One type of improvement includes making safer packaging cell lines that are generated by deletions in the 3' Long Terminal Repeat (LTR). Other improvements include increasing the host range by replacement of one viral env gene with that of another viral env gene thereby creating a hybrid producer line that generates pseudotyped helper viruses. More specifically HIV has been given an extended host cell range by pseudotyping with the unrelated viruses VSV and HSV (Zhu et al., J. Aids, 3:215 – 219 (1990) and Naldini et al., Science, 272:263-267, (1996)). Further improvements have been made by the use of minimum viral coding regions on the vector. Lentivirus can infect nondividing cells and this property is especially useful for gene delivery. Additionally, most packaging cell lines currently in use have been transfected with separate plasmids each containing one of the necessary coding sequences so that

multiple recombination events would be necessary before replication competent virus can be produced. PCT publication WO 01/44458, which is hereby incorporated by reference, describes examples of a BIV based lentiviral vector system.

In another embodiment, treatment of type-2 diabetes, obesity, and related conditions is achieved by in vivo expression of glucagon-like peptide 1 (GLP-1), and/or glucose-dependent insulinotropic peptide (GIP), preferably combined with concurrent administration of DPP-IV inhibitors. GLP-1, GIP, or a combination of both GLP-1 and GIP are delivered to the animal via gene delivery vectors. These vectors are used to induce insulin secretion and can be used to study diabetes, obesity and related diseases. These vectors may also be used to treat diabetes, obesity and related diseases. Preferable vectors include adenoviral vectors (preferably lacking all viral genes, i.e. high capacity or gutless), lentiviral vectors (e.g. HIV, BIV-based), and adeno-associated virus (AAV) vectors.

Signal sequences can be used in the GLP-1 or GIP expression cassette. Two preferred signal sequences are the murine Igκ signal and the glia monster exendin signal (Chen and Drucker, 1996). In addition, because GLP-1 and GIP are such small peptides (less than 40 aa), to achieve efficient translation, signal cleavage, and secretion, it is preferable to lengthen the pro-peptide. This can be achieved by the addition of amino acids at the amino terminal, downstream of the signal peptide. The pro-sequence can be specifically cleaved from the GLP-1 or GIP coding region by a protease. A preferred protease is furin, when a furin cleavage site is engineered into the pro-sequence.

One embodiment has the expression of GLP-1 and/or GIP controlled by a constitutive promoter. Non-limiting examples of constitutive promoters include the cytomegalovirus (CMV) promoter, the Rous sarcoma long- terminal repeat (LTR) sequence, and the SV40 early gene promoter.

Another embodiment has the expression of GLP-1 and/or GIP controlled by an inducible promoter. One example of a controlled promoter system is the Tet-On ™ and Tet-Off ™ systems currently available from Clontech (Palo Alto, CA). This promoter system allows the regulated expression of the transgene controlled by tetracycline or tetracycline derivatives, such as doxycycline. This system could be used to control the expression of GLP-1 and/or GIP. Other regulatable promoter systems are described in the PCT publications WO 01/30843 and WO 02/06463, which are hereby incorporated by reference in their entirety.

A significant limitation to potential GLP-1 and GIP therapy in diabetic patients is the short biological half-life that makes protein replacement therapy problematic. The short half-life is due to the cleavage of GLP-1 and GIP by DPP-IV. Two alternative strategies have been employed to circumvent this problem. For GLP-1, variants derived from different species, such as the glia monster exendin peptide (Hughes, 1998; Grieg et al., 1999; Young et al., 1999), or derivatives of the mammalian GLP-1, that are not readily degraded by DPP-IV (Burcelin et al., 1999; Gallwitz et al., 2000), have been generated and characterized.

Another embodiment of the invention includes vectors including GLP-1 variants that have increased resistance to DPP-IV degradation. These variants include, but are not limited to, those known in the art. For example, those described by Hughes (1998); Grieg et al. (1999); and Young et al., (1999).

Alternatively, an approach to inhibit the action of DPP-IV in vivo, thereby functionally increasing the half-life of GLP-1 and/or GIP, has been employed (reviewed by Holst and Deacon, 1998). It is not expected that inhibition of DPP-IV would have long-term negative or toxic effects on the treated patient, since DPP-IV-deficient rats appear completely unaffected by this deficiency (Pederson et al., 1996). Animal studies have been reported demonstrating that administration of DPP-IV inhibitors, such as isoleucine thiazolidide or valine-pyrrolidide, leads to an improvement in glucose tolerance (Pederson et al., 1998, Pauly et al., 1999; Deacon et al., 2001). The demonstration of the utility of these compounds has been described (Balkan et al., 1999). Non-limiting examples of DPP-IV inhibitors are described in US 6,110,949, US 6,107,317, US 6,011,155, US 6,172,081, and US 6,166,063, which are hereby incorporated by reference in their entirety.

"GLP1" is defined as GLP1(7-37), GLP1(7-36), or variants thereof.

"Expression cassette" is defined as a polynucleotide, when introduced to the desired cell, will express the encoded amino acid.

"Secretion signal" is defined as an amino acid sequence, or an amino acid sequence encoded by a polynucleotide, which, when present at the amino terminus of a polypeptide, functions to mediate polypeptide secretion.

"Administration" is defined as in vivo delivery of the gene transfer vehicle.

"Gene delivery vector" is defined as a gene transfer vehicle capable of transferring the gene of interest following in vivo or in vitro administration.

"Exendin gene" is defined as any nucleotide encoding for the glia monster exendin peptide (Seq ID:12), including SEQ ID:11.

Furin cleavage site is defined as the sequence Arg-Xaa-Lys-Arg (SEQ ID:18), where Xaa is preferentially Thr.

"Igk gene" is defined as the immunoglobulin kappa gene, including the signal sequence.

"Adenovirus vector" or "adenoviral vector" (used interchangeably) is defined as a polynucleotide comprising all or a portion of an adenoviral genome.

Table 1

GLP-1 peptides and derivatives

GLP-1 (7-37) (SEQ ID NO:1 and 2)

5'-CAC-GCT-GAA-GGG-ACC-TTT-ACC-AGT-GAC-GTA-AGT-TCT-TAT-TTG-GAA-GGC-
H A E G T F T S D V S S Y L E G

CAA-GCT-GCC-AAG-GAA-TTC-ATT-GCT-TGG-CTG-GTG-AAA-GGC-CGA-GGA-3'
Q A A K E F I A W L V K G R G

GLP-1 (7-36) (SEQ ID NO:3 and 4)

5'-CAC-GCT-GAA-GGG-ACC-TTT-ACC-AGT-GAC-GTA-AGT-TCT-TAT-TTG-GAA-GGC-
H A E G T F T S D V S S Y L E G

CAA-GCT-GCC-AAG-GAA-TTC-ATT-GCT-TGG-CTG-GTG-AAA-GGC-CGA-3'
Q A A K E F I A W L V K G R

GLP-1 Variants

GLP-1 (7-37) (A-8-G) (SEQ ID NO:5 and 6)

5'-CAC-GGT-GAA-GGG-ACC-TTT-ACC-AGT-GAC-GTA-AGT-TCT-TAT-TTG-GAA-GGC-
H G E G T F T S D V S S Y L E G

CAA-GCT-GCC-AAG-GAA-TTC-ATT-GCT-TGG-CTG-GTG-AAA-GGC-CGA-GGA-3'
Q A A K E F I A W L V K G R G

GLP-1 (7-37) (A-8-S) (SEQ ID NO:7 and 8)

5'-CAC-TCT-GAA-GGG-ACC-TTT-ACC-AGT-GAC-GTA-AGT-TCT-TAT-TTG-GAA-GGC-
H S E G T F T S D V S S Y L E G

CAA-GCT-GCC-AAG-GAA-TTC-ATT-GCT-TGG-CTG-GTG-AAA-GGC-CGA-GGA-3'
Q A A K E F I A W L V K G R G

GLP-1 (7-37) (A-8-T) (SEQ ID NO:9 and 10)

5'-CAC-ACT-GAA-GGG-ACC-TTT-ACC-AGT-GAC-GTA-AGT-TCT-TAT-TTG-GAA-GGC-
H T E G T F T S D V S S Y L E G

CAA-GCT-GCC-AAG-GAA-TTC-ATT-GCT-TGG-CTG-GTG-AAA-GGC-CGA-GGA-3'
Q A A K E F I A W L V K G R G

Table 2

Exendin Nucleotide And Amino Acid Sequence (SEQ ID NO:11 and 12)

5'-CAT-GGT-GAA-GGA-ACA-TTT-ACC-AGT-GAC-TTG-TCA-AAA-CAG-ATG-GAA-
H G E G T F T S D L S K Q M E

GAG-GAG-GCA-GTG-CGG-TTA-TTT-ATT-GAG-TGG-CTT-AAG-AAC-GGA-GGA-CCA
E E A V R L F I E W L K N G G P

AGT-AGC-GGG-GCA-CCT-CCG-CCA-TCG-3'
S S G A P P P S

GIP Nucleotide And Amino Acid Sequence (SEQ ID NO:13 and 14)

5'-TAC-GCG-GAA-GGG-ACT-TTC-ATC-AGT-GAC-TAC-AGT-ATT-GCC-ATG-GAC-
Y A E G T F I S D Y S I A M D

AAG-ATT-CAC-CAA-CAA-GAC-TTT-GTG-AAC-TGG-CTG-CTG-GCC-CAA-AAG-GGG-
K I H Q Q D F V N W L L A Q K G

AAG-AAG-AAT-GAC-TGG-AAA-CAC-AAC-ATC-ACC-CAG-3'
K K N D W K H N I T Q

Table3

Signal Sequences

Exendin signal (SEQ ID NO:15 and 16)

5'-ATG-AAA-ATC-ATC-CTG-TGG-CTG-TGT-GTT-TTT-GGG-CTG-TTC-CTT-GCA-ACT-
M K I I L W L C V F G L F L A T

TTA-TTC-CCT-ATC-AGC-TGG-CAA-ATG-CCT-GTT-GAA-TCT-GGG-TTG-TCT-TCT-
L F P I S W Q M P V E S G L S S

GAG-GAT-TCT-GCA-AGC-TCA-GAA-AGC-TTT-GCT-TCG-AAG-ATT-AAG-CGA-3'
E D S A S S E S F A S K I K R

IgK signal (SEQ ID NO:17 and 18)

5'-ATG-GAG-ACA-GAC-ACA-CTC-CTG-CTG-TGG-GTA-CTG-CTG-CTC-TGG-GTT-CCA-
M E T D T L L L W V L L L W V P

GGT-TCC-ACT-GGT-GAC-3'
G S T G D

Examples

Example 1

GLP-1(7-37)-6xHis Expression Cassette With Igk Signal Sequence

First the GLP-1 sequence along with the signal sequence is designed. Then using the properly designed oligos, a PCR-based gene synthesis method (Withers-Martinez et al., 1999) is used to construct the signal-sequence-GLP-1 (ssGLP-1) construct. For example, the oligos listed in Table 4 are utilized to construct a ssGLP-1 construct containing a murine IgK signal sequence (Seq ID:17 and 18, nucleotide and amino acid sequence, respectively).

Table 4

Ig kappa signal, GLP-1(7-37) with 6xHis-tag - Oligos for Gene Synthesis

Upper Strand

Oligo Label	Oligo Sequence	SEQ ID NO:
UG1	5'-ATACAGCTCGAGACGCGTAT-3'	19
UG2	5'-GGAGACAGACACACTCCTGCTGTGGGTACTGCTGCTCTGG-3'	20
UG3	5'-GTTCCAGGTTCCACTGGTGACGCGCACGCTGAAGGGACCT-3'	21
UG4	5'-TTACCAGTGACGTAAGTTCTTATTTGGAAGGCCAAGCTGC-3'	22
UG5	5'-CAAGGAATTCATTGCTTGGCTGGTGAAAGGCCGAGGACAT-3'	23
UG6	5'-CATCATCATCATCATTGATCTAGAGTCGACGTAATCCGCT-3'	24

Lower Strand

Oligo Label	Oligo Sequence	
LG1	5'-AGCGGATTACGTCGACTCTA-3'	25
LG2	5'-GATCAATGATGATGATGATGATGTCCTCGGCCTTTCACCA-3'	26
LG3	5'-GCCAAGCAATGAATTCCTTGGCAGCTTGGCCTTCCAAATA-3'	27
LG4	5'-AGAACTTACGTCACTGGTAAAGGTCCCTTCAGCGTGCGCG-3'	28
LG5	5'-TCACCAGTGGAACCTGGAACCCAGAGCAGCAGTACCCACA-3'	29
LG6	5'-GCAGGAGTGTGTCTGTCTCCATACGCGTCTCGAGCTGTAT-3'	30

Gene synthesis is performed with the oligos in Table 4 as described in Withers-Martinez et al., 1999. Oligos were ordered from Life Technologies (Frederick, MD). Briefly, all oligos are diluted to a concentration of 100μM. Equal volumes of each oligo are combined, creating a solution which is further diluted to a concentration of 450nM. The first PCR cycle (gene assembly step) is carried out using Pfu polymerase (Stratagene, La Jolla, California). The gene assembly PCR reaction is performed by running the following reaction.

Reaction Mixture	PCR Cycle
5 ul Oligo Mix (450 nM)	Step#1 94°C - 60 seconds
5 ul 10x Cloned Pfu PCR Buffer (Stratagene)	Step#2 94°C - 30 seconds
1 ul dNTPs (10 mM each)	Step#3 52°C - 30 seconds
38 ul Water	Step#4 72°C - 120 seconds
1 ul Pfu Polymerase	Step#5 Goto step#2 26 times
	Step#6 72°C - 10 Minutes

The gene amplification PCR is performed by running the following PCR reaction.

Reaction Mixture	PCR Cycle		
5 ul Gene assembly reaction	Step#1	94°C -	60 seconds
5 ul 10x Cloned Pfu PCR Buffer (Stratagene)	Step#2	94°C -	45 seconds
1 ul dNTPs (10 mM each)	Step#3	51.4°C -	45 seconds
37 ul Water	Step#4	72°C -	5 minutes
1 ul Pfu Polymerase	Step#5	Goto step#2 25-times	
0.5 ul UG1 primer 100 μM	Step#6	72°C -	10 minutes
0.5 ul LG1 primer 100 μM			

The gene amplification PCR is analyzed by gel electrophoresis and the desired PCR fragment is isolated from the gel using Stratagene's Gel Extraction Kit (Stratagene, La Jolla, California). The isolated fragment is digested with XhoI and XbaI and ligated into pCi (Promega, Madison, WI) that is previously digested with XhoI and XbaI, creating the plasmid pCiGLP1IgK+Ala (Figure 1A). This GLP-1 expression cassette consists of, from 5' to 3', a promoter operably linked to a sequence encoding GLP-1, a signal sequence to allow secretion of GLP-1 from transduced cells, a coding region of GLP-1 with a 6xHistidine tag and a polyadenylation signal. The 6x Histidine tag is utilized for purification of the GLP-1 protein. It is not necessary for expression of GLP-1 or utilization of the invention.

Example 2

GLP-1(7-37)-6xHis Expression Cassette With Exendin Signal Sequence

This construct is cloned using the same procedure as described in Example 1. The procedure is performed with the oligos from Table 5 and the resulting PCR fragment is cloned into pCi (Promega, Madison, WI) as described in Example 1. The resulting plasmid is designated pCiGLP1Ext (Figure1B)

Table 5

Exendin signal, GLP-1(7-37) with 6xHis-tag - Oligos for Gene Synthesis

Upper Strand

Oligo Label	Oligo Sequence	SEQ ID NO:
UG1	5'-ATACAGCTCGAGACGCGTAT-3'	19
UG2-EX	5'-GAAAATCATCCTGTGGCTGTGTGTTTTTGGGCTGTTCCTT-3'	31
UG3-EX	5'-GCAACTTTATTCCCTATCAGCTGGCAAATGCCTGTTGAAT-3'	32
UG4-EX	5'-CTGGGTTGTCTTCTGAGGATTCTGCAAGCTCAGAAAGCTT-3'	33
UG5-EX	5'-TGCTTCGAAGATTAAGCGACACGCTGAAGGGACCT-3'	34
UG4	5'-TTACCAGTGACGTAAGTTCTTATTTGGAAGGCCAAGCTGC-3'	22
UG5	5'-CAAGGAATTCATTGCTTGGCTGGTGAAAGGCCGAGGACAT-3'	23
UG6	5'-CATCATCATCATCATTGATCTAGAGTCGACGTAATCCGCT-3'	24

Lower Strand

Oligo Label	Oligo Sequence	
LG1	5'-AGCGGATTACGTCGACTCTA-3'	25
LG2	5'-GATCAATGATGATGATGATGATGTCCTCGGCCTTTCACCA-3'	26
LG3	5'-GCCAAGCAATGAATTCCTTGGCAGCTTGGCCTTCCAAATA-3'	27
LG4-EX	5'-AGAACTTACGTCACTGGTAAAGGTCCCTTCAGCGT-3'	35
LG5-EX	5'-GTCGCTTAATCTTCGAAGCAAAGCTTTCTGAGCTTGCAGA-3'	36
LG6-EX	5'-ATCCTCAGAAGACAACCCAGATTCAACAGGCATTTGCCAG-3'	37
LG7-EX	5'-CTGATAGGGAATAAAGTTGCAAGGAACAGCCCAAAAACAC-3'	38
LG8-EX	5'-ACAGCCACAGGATGATTTTCATACGCGTCTCGAGCTGTAT-3'	39

Example 3

GLP-1(7-37) Expression Cassette With Igk Signal Sequence and a Propeptide Sequence

The plasmid, pCi-nGLP1IgkplfHis (Figure 1C), contains the CMV promoter, a chimeric intron, the murine Igk signal sequence (isolated from pSecTag2, InVitrogen, Carlsbad, CA), a 30 aa prosequence isolated from the pSecTag2 polylinker, a furin cleavage

site (consensus Arg-Thr-Lys-Arg (SEQ ID:18); Groskreutz et al., 1994), the GLP-1 coding region, a 6-His tag (isolated from pSecTag2), and the Sv40 late polyadenylation signal. This plasmid is constructed using the same procedure as described in Example 1, with the following few exceptions. The gene assembly PCR is performed with the oligos from Table 6. The concentration of Oligos Mix is 348 nM. The gene amplification PCR is performed with a temperature of 62.4°C in step #3. The resulting PCR fragment is cloned into pCi-neo (with an SV40 origin of replication). The resulting plasmid is designated pCi-nGLP1IgkplfHis (Figure 1C).

Table 6

IgK signal, Propeptide sequence, Furin cleavage site, GLP-1(7-37) with 6xHis-tag Oligos for gene synthesis

Upper Strand **SEQ ID NO:**

Oligo Label	Oligo Sequences	
UG1	5'-ATACAGCTCGAGACGCGTAT-3'	19
UG2	5'-GGAGACAGACACACTCCTGCTGTGGGTACTGCTGCTCTGG-3'	20
UGF3	5'-GTTCCAGGTTCCACTGGTGACGCGGCCCAGCCGGCCAGGCGCGCCG-3'	40
UGF4	5'-TACGAAGCTTGGTACCGAGCTCGGATCCACTCCAGTGTGGTGGAAT-3'	41
UGF5	5'-TCTGCAGATATCCAGCACAAGGACCAAGCGGCACGCTGAAGGGACCT-3'	42
UG4	5'-TTACCAGTGACGTAAGTTCTTATTTGGAAGGCCAAGCTGC-3'	22
UG5	5'-CAAGGAATTCATTGCTTGGCTGGTGAAAGGCCGAGGACAT-3'	23
UG6	5'-CATCATCATCATCATTGATCTAGAGTCGACGTAATCCGCT-3'	24

Lower Strand

Oligo Label	Oligo Sequences	
LG1	5'-AGCGGATTACGTCGACTCTA-3'	25
LG2	5'-GATCAATGATGATGATGATGATGTCCTCGGCCTTTCACCA-3'	26
LG3	5'-GCCAAGCAATGAATTCCTTGGCAGCTTGGCCTTCCAAATA-3'	27
LGF4	5'-AGAACTTACGTCACTGGTAAAGGTCCCTTCAGCGTGCCGCTTG-3'	43
LGF5	5'-GTCCTTGTGCTGGATATCTGCAGAATTCCACCACACTGGAGTGGATC-3'	44
LGF6	5'-CGAGCTCGGTACCAAGCTTCGTACGGCGCGCCTGGCCGGCTGGGCC-3'	45
LGF7	5'-GCGTCACCAGTGGAACCTGGAACCCAGAGCAGCAGTACCCACA-3'	46
LG6	5'-GCAGGAGTGTGTCTGTCTCCATACGCGTCTCGAGCTGTAT-3'	30

Example 4

Analysis of In vitro GLP-1 expression

The GLP-1 plasmid constructs are tested in vitro for GLP-1 expression. For this analysis, 30 ug of the GLP-1 expression plasmids (pCi-nGLP1IgkplfHis, pCiGLP1Ext), are transiently transfected into 293T cells using the $CaPO_4$ transfection method. 48 hrs after

transfection, media is collected and assayed for the presence of GLP-1 by ELISA (Linco Research Inc, St. Charles, Missouri). The standard curve, using purified GLP-1 (7-36 amide) at concentrations ranging from 0-100 pM, and the OD readings are displayed in Table 7 (R^2=0.9919). Using this standard curve, the concentrations of GLP-1 expressed from the plasmid constructs is extrapolated (Table 8). Both GLP-1 expression constructs express measurable amounts of GLP-1. The exendin plasmid (pCiGLP1Ext, designated as exendin in Table 8) expresses the highest levels of GLP-1, approximately 310 pM, while the Igκ plasmid (pCi-nGLP1IgkplfHis, designated as GLP1-1 and GLP1-2 in Table 8) expresses approximately 60 pM.

GLP1(7-36) amide Standards (pM)	ELISA Reading Fluor. Units
0	13.5
2	18.5
5	25.5
10	29.0
20	48.5
50	165.5
100	348.5

Table 7. The OD readings using purified GLP-1 (7-36 amide) peptide in the GLP-1 ELISA (Linco Research Inc.). The standard concentrations ranged from 0-100 pM. R^2=0.9919

Sample	ELISA Reading Fluor. Units	GLP1 Concentration (pM)
GLP1-1+	**227**	**66.12**
GLP1-1-	**138**	**40.43**
GLP1-2+	**184**	**53.71**
GLP1-2-	**299**	**86.9**
Exendin +	**1132**	**327.35**
Exendin -	**1001**	**289.54**
GFP+	**16**	**5.21**
GFP-	**12**	**4.06**

Table 8. GLP-1 ELISA results using supernatant collected from 293T cells transfected with the GLP-1 expression cassettes. The GLP-1 concentrations were calculated from the standard curve (Table 7). GLP1-1, and GLP1-2, designate media isolated from cells transfected with pCi-nGLP1IgkplfHis. The 1 and 2 designations indicate two different clones

of the pCi-nGLP1IgkplfHis plasmid. Exendin designates media isolated from cells transfected with (pCiGLP1Ext). The (+) or (-) indicates the inclusion, or absence of serum in the media, respectively.

These constructs contain a 6-His tag, which is added to aid in protein purification for subsequent NH_2-terminal sequence analysis. It is possible that this tag interferes with the GLP-1 ELISA. To directly test whether this affects the accuracy of the the GLP-1 expression data, various GLP-1 peptides are synthesized, including GLP-1(7-37) with a 6xHis tag, GLP-1 (7-36) amide, GLP-1 (7-36, Ser 8 mutation) amide, GLP-1 (7-36) without amide, and GLP-1 (7-37). Interestingly, the GLP-1 (7-36) amide, and GLP-1 (7-36) without amide show predicted quantitation using the Linco ELISA (Table 9). However, GLP-1 (7-37) GLP-1(7-37) with a 6xHis tag, and GLP-1 (7-36, Ser 8 mutation), show less efficient detection (Table 9). These data suggest that the GLP-1 peptide present in the conditioned media may actually be more concentrated than indicated by the ELISA data.

Peptide	Concentration	ELISA Reading extrapolated concentration (pM)
GLP1(7-36) Amide	10nM	1117
	1nM	109
	100pM	15
	10pM	9
Ser8-GLP1(7-36) Amide	10nM	302
	1nM	17
	100pM	11
	10pM	11
GLP1(7-36) Without amide	10nM	1143
	1nM	117
	100pM	14
	10pM	10
GLP1(7-37)	10nM	734
	1nM	237
	100pM	22
	10pM	21
GLP1(7-37) 6xHIS	10nM	449
	1nM	13
	100pM	19
	10pM	16

Table 9. GLP-1 ELISA results using various synthesized peptides. The type of GLP-1 peptide is displayed in the first column, the actual peptide concentration is displayed in the middle column, and the calculated value is displayed in the last column.

Example 5

The use of an inducible gene expression system will allow the regulation of GLP-1 and GIP in a reversible manner. Several inducible systems are currently available. For example, the Tet-On ™ and Tet-Off ™ systems currently available from Clontech (Palo Alto, CA) allow the regulated expression of the transgene controlled by tetracycline or tetracycline derivatives, such as doxycycline. The Tet-Off system uses the tetracycline-controlled transactivator (tTA), which is composed of the tet repressor protein (TetR) and the VP16 activation domain. tTA activates transcription in the absence of tetracycline. The Tet-On system uses the reverse tetracycline-controlled transactivator (rtTA) and activates transcription in the presence of tetracycline. Both systems use the tetracycline-response element (TRE) which contains 7 repeats of the tet operator sequence, and the target gene, such as GLP-1 or GIP. tTA or rtTA bind to the TRE, activating transcription of the target gene.

To generate tet-controlled GLP-1 and GIP expression cassettes, the expression cassettes described above are cloned into the pTRE2 plasmid (Clontech, Palo Alto, CA) into the multiple cloning sequence. These plasmids, pTRE2-GLP-1 and pTRE2-GIP, in addition to the expression cassette containing the Tet-On or Tet-Off expression elements (contained in plasmid pTet-On or pTet-Off, Clontech), are inserted into the gutless vector plasmid, pGTI14 or pGTI15, and a gutless vector encoding both expression cassettes is generated (Figure 2) by methods known to those skilled in the art.

Example 6

Generation and production of gutless adenoviral vectors encoding GLP-1 and/or GIP expression cassettes

The GLP-1 and GIP-1 gutless adenoviral vectors are generated in the following manner. To make the gutless vector plasmids, bp 1-384 of Ad5, which contains the ITR and viral packaging signal is PCR amplified using the following oligonucleotides:

#1 (5'- GCAGGTACCTTAATTAACATCATCAATAATATACCTTATTTTG 3') (SEQ ID NO:47)

#2 (5'- GAACTGCAGGTCTCCACGTAAACGGTCAAAG 3') (SEQ ID NO:48)

The adenoviral sequences are displayed in bold. Pac I and Pst I restriction enzyme sites are engineered in oligonucleotides 1 and 2, respectively. Next, 420 bp of the right end of Ad5 (bp 35,516 to 35,935) is PCR amplified using oligonucleotide #3 (5' GAACTGCAGGGCAGCCTAACAGTCAGCCTTACC 3') (SEQ ID NO:49), encoding a Pst I site, and oligonucleotide #1 (above). The PCR products are digested with Pst I and inserted into pBluescript II (Stratagene, La Jolla, CA) digested with Ssp I and Pvu II in a three-way ligation, to construct pGTL10a. Therefore, pGTI10a contains both termini of the Ad5 genome bordered by Pac I sites. In the next step, pGTL11a is generated from pGTL10a by the removal of 125 bp from the right terminal fragment including the cap site and TATA box from the promoter region of E4, Ad5 bps 35,516 to 35,640. Next, pGTL14 and pGTL15 are generated from pGTL10a and pGTL11a, respectively, following insertion of 21.9 kb human synuclein intronic region stuffer sequence. The final gutless vector plasmids are generated by insertion of the GLP-1 and/or the GIP expression cassettes into pGTL14 and/or pGTL15. A 4 kb DNA stuffer sequence, derived from human genomic DNA C346 (Sandig et al., 2000), which is isolated from human cells by PCR amplification is inserted into pGTL14 and pGTL15 to generated pGTL14C346 and pGTL15C346, respectively. The final gutless vector plasmids are generated by insertion of one or several GLP-1 and/or the GIP expression cassettes into pGTL14C346 and/or pGTL15C346. Examples of these resulting plasmids are pGTL14C346GLP-1 and pGTL15C346GLP-1, respectively (Figures 3).

The helper virus, Av1nBgflx, encodes a β-galactosidase (β-gal) marker gene in a viral backbone lacking E1 and E3. Av1nBgflx is generated by cotransfecting two plasmids, pAV6alxirsvnbgspa and pSQ1 into PER.C6 cells (Fallaux et al., 1998) following standard protocols. The plasmid, pAV6alxirsvnbgspa is constructed in several steps. First, pAvS6 (Gorziglia et al., 1996), encoding Ad5 bp 1 to 393 and 6246 to 3509 is modified by insertion of a 34 bp long loxP site at Ad5 bp 191, to produce pAV6alxa. Next, a second loxP site is inserted into pAV6alxa at Ad5 bp 393, to generate pAV6alxi. In the final step, the β-gal expression cassette, encoding an RSV promoter, nuclear localized β-gal, and a synthetic poly(A) signal, is inserted into pAV6alxi to generate pAV6alxirsvnbgspa. The plasmid pSQ1 contains the majority of the Ad5 genome excluding the E1 and E3 regions, Ad5 bp position

3,329 to 28,591 and 30,470 to 35,935. Viral genome integrity is confirmed by restriction analysis of DNA isolated from CsCl-purified vector.

Gutless vector generation from plasmid, and large scale vector production is performed using C7-Cre cells (Hartigan-O'Connor, et al., 1999), which are a 293-based cell line, further modified to express the Ad5 E2b proteins (pTP and Pol) and Cre recombinase of P1 bacteriophage. Gutless vector sequences are liberated from pGTI.14GLP-1 and pGTL15GLP-1 by Pac I digestion and transfected into sub-confluent monolayers of C7-Cre cells in a six well dish using the Profection calcium phosphate transfection method (Promega, Madison, WI). Twelve to sixteen hours after transfection, the cells are infected with helper virus at a dose of 100 particles/cell. Cells are harvested when complete cytopathic effect (CPE) is observed. Cells are subjected to three cycles of freeze-thaw, and the lysate is used to infect one 150 mm dish of C7-Cre cells with helper virus supplementation, 100 particles/cell. When CPE is complete (approximately 48 hrs after helper virus addition), the cell lysate is used to infect 10 x 150-mm plates of C7-Cre cells with helper virus supplementation. Gutless vectors are purified by CsCl gradient centrifugation. CsCl purified gutless vector preparations are subsequently used as seedlots for vector production. Using the gutless vector seed lot, C7-Cre cells are infected with 30 particles of gutless vector/cell and with helper virus at 100 particles /cell.

To confirm the correct vector is isolated, DNA from purified gutless vectors is extracted and digested with BamHI and the ends of the resulting DNA fragments are labelled with α-^{33}P-dGTP using Klenow fragment. The restriction enzyme fragments are then resolved on agarose gel stained with ethidium bromide and photographed. Next, the DNA fragments are transferred from agarose gel to Hybond-N and subjected to autoradiography. The bands from the gel are observed to see if they match the expected sizes for the correct gutless vector.

Hexon Taqman real-time PCR assay: PCR primers and a Taqman probe specific to adenovirus hexon sequences are designed using Primer Express software V. 1.0 (Applied Biosystems, Foster City, CA). Primer and probe sequences are:

hexon forward primer: 5' CTTCGATGATGCCGCAGTG-3' (SEQ ID NO:50);

hexon reverse primer: 5' GGGCTCAGG TACTCCGAGG-3' (SEQ ID NO:51);

hexon probe: 5' FAM-TTACATGCACATCTCGGGCCAGGAC-TMRA-3' (SEQ ID NO:52).

Amplification is performed in a reaction volume of 50 ul under the following conditions: 1,500,000 copies of vector DNA, 1X Taqman universal PCR master mix (Applied Biosystems), 600 nM forward primer, 900 nM reverse primer and 100 nM hexon probe. Thermal cycling conditions are: 2 minutes incubation at 50^0C, 10 minutes at 95^0C, followed by 35 cycles of successive incubation at 95^0C for 15 seconds and 600C for 1 minute. Data is collected and analysed using 7700 Sequence Detection System software v.1.6.3. (Applied Biosystems). Quantification of adenovirus copy number is performed using standard curve consisting of dilutions of gutless adenovirus DNA from 1,500,000 copies to 15 copies. The percent helper virus is calculated as the average hexon copy number from three PCR replicates divided by 1,500,000 total vector copies.

For quantitation of the β-gal-encoding helper virus, 1.75×10^5 AE1-2a cells (Gorziglia et al., 1996) are seeded in 24-well plates. The cells are infected with multiplicities of infections of 2000, 1000, 500, 250 and 125 viral particles/cell. Twenty-four hours following infection, the cells are fixed and stained with X-gal (Smith et al., 1993) and beta-gal positive cells are counted.

The vector preparations are checked for the presence of replication-competent adenovirus (RCA) contamination by PCR directed at E1a sequences (Tolstoshev et al., 1994). All vector concentrations are determined by spectrophotometric analysis (Mittereder et al., 1996). Titers are given as viral particles per milliliter.

The gutless vectors described in Examples 6A-6C are generated as described above in Example 6.

Example 6A

Generation of AGV-C7-GLP

The gutless adenoviral vector, AGV-C7-GLP contains a GLP-1 expression cassette composed of the exendin signal sequence, GLP-1 coding region, and SV40 early polyadenylation signal, driven the by the inducible regulated promoter. This promoter has been described by Xu et al. (2001). The plasmid, pBV2.CGE.C7.GLP-1Ex (Figure 4A), used to generate the gutless vector AGV-C7-GLP, is constructed in the following manner. First, the C7, inducible promoter region is isolated from pAGVC7endo1c (Figure 4B) using NheI and Pme I. The exendin/GLP-1 coding region is isolated from pCiGLP1Ex (described in Example 2; Fig. 1B), using Nhe I and Cla I. Both fragments are combined with pGTL24aPL2 (Figure 4C) to generate pGTI.APL2.CGE.C7.GLP-1Ex (Figure 4D). Then,

pGTLAPL2.CGE.C7.GLP-1Ex is digested with PacI to liberate the plasmid backbone and combined with PmeI and MluI digested pBV2 (Figure 4E). The final gutless vector is generated by homologous recombination in BJ5138 E.coli as described by Toietta et al. (2002).

The gutless vector, AGV-C7-GLP is generated following transfection of the plasmid into C7-Cre cells (Reddy et al., 2002) which are a 293-based cell line, further modified to express the Ad5 E2b proteins (pTP and Pol) and Cre recombinase of P1 bacteriophage. Gutless vector sequences are liberated from pBV2.CGE.C7.GLP-1Ex by digestion with Pac I and 50 ug of DNA per plate is transfected into sub-confluent monolayers of C7-Cre cells in 3-x15 cm tissue culture plates using the Profection calcium phosphate transfection method (Promega, Madison, WI). Twelve to sixteen hours after transfection, the cells are infected with the helper virus, Av1S4BflxFE3 (Sakhuja et al., 2002) (in press) at a dose of 100 particles/cell. Cells are harvested when complete cytopathic effect (CPE) is observed. Cells are subjected to three cycles of freeze-thaw, and the lysate is used to infect 10 x 15 cm plates of C7-Cre cells with helper virus supplementation. Gutless vectors are purified by CsCl gradient centrifugation. CsCl purified gutless vector preparations are subsequently used as seedlots for vector production. Using the gutless vector seed lot, C7-Cre cells are infected with 30 particles of gutless vector/cell with helper virus at 100 particles /cell. A CsCl gradient is used to purify the AGV-C7-GLP gutless vector. A total yield of 2×10^{12} particles in the 30x15 cm plates was obtained. DNA from this purified gutless vector is extracted and digested with Not I and restriction enzyme fragments are resolved on an agarose gel stained with ethidium bromide. The expected restriction enzyme pattern after Not I digestion for the gutless vector is: 19515, 5003, 3217, 415, and 42, and for the helper virus: 12193, 7932, 6428, 5001, 2589, 1931, 960, and 326. The identity and integrity of the gutless vector genome are verified by restriction digest.

Example 6B

Generation of AGV-CMV-GLP

The gutless adenoviral vector, AGV-CMV-GLP contains a GLP-1 expression cassette composed of the exendin signal sequence, GLP-1 coding region, and SV40 early polyadenylation signal, driven by the CMV promoter. The plasmid, pBV2.CMV.GLP-1Ex (Figure 5A), used to generate the gutless vector AGV-CMV-GLP, is constructed in the following manner. First, the plasmid, pGTLAPL2.CMV.GLP-1Ex (Figure 5B) is constructed

by digesting pCiGLP1Ex with Bgl II, filling in the ends, and digesting with ClaI and ligating to pGTL24APL2 digested with SmaI and NarI. Then pGTLAPL2.CMV.GLP-1Ex, is digested with Pac I to liberate the plasmid backbone and combined with PmeI and MluI digested pBV2 (Figure 4E). The final gutless vector is generated by homologous recombination in BJ5138 *E.coli* as described by Toietta *et al.*, (2002).

The AGV-CMV-GLP gutless vector is generated and propagated as described above. Supernatant collected from cells propagating the AGV-CMV-GLP gutless vector, collected from the 10-plate CVL stage, is assessed for GLP-1 expression by ELISA (Linco Research Inc., St. Charles, Missouri), as described. The data are displayed in Table 10.

Sample	ELISA Reading	GLP-1 (pM)
GLP-1 Standards (pM)		
0	27	
2	54	
5	84	
10	127	
20	305	
50	942	
100	1805	
Quality Controls		
#1 (Expected range 4-9 pM)	87	5.3
#2 Expected range 44-92 pM)	1200	66.2
Samples : CMV –GPLP-1 AVG		
#1	73	4.22
#2	57	3.67
#3	47	3.1

Table 10. GLP-1 ELISA results from CVL of C7 cre cells infected with AGV-CMV-GLP. The CVL was collected from one 150mm plate 48hrs following the initial stage of virus production.200ul of CVL was used for each well.Data represents the mean values of three wells. GLP-I concentrations were calculated from a standard curve.

Example 6C
Generation of AGV-ALB-GLP

The gutless adenoviral vector, AGV-ALB-GLP contains a GLP-1 expression cassette composed of the exendin signal sequence, a liver-specific albumin promoter operably linked to a GLP-1 coding region, and SV40 early polyadenylation signal. First, the albumin promoter and heterologous intron from the Apo A1 gene, is isolated by PCR amplification of the plasmid, pAvALAPH81 (Connelly et al., 1996). The oligonucleotide primers for the PCR amplification are as follows: 5' GATATGTTTAAACACGCGTGCTATGACC 3' (SEQ ID NO:53) with the addition of a Pme I site (bold underline), and 5' CCTGGGCTAGCAGGAGAAGAAGGGC 3' (SEQ ID NO:54) with the addition of an NheI site (bold underline). The PCR fragment is digested with PmeI and NheI and combined with NheI and ClaI digested pCiGLP1Ex and inserted into pGTL24ALP2 digested with PmeI and NarI to obtain pGTLAPL2.ALB.GLP-1EX (Figure 6A). Next, pGTLAPL2.ALB.GLP-1EX is digested with PacI to liberate the plasmid backbone and combined with PmeI and MluI digested pBV2, to create pBV2.ALB.GLP-1EX (Figure 6B). The final gutless vector is generated by homologous recombination in BJ5138 *E.coli* as described by Toietta *et al.*, (2002).

Example 7

Generation and production of BIV vectors encoding GLP-1 and/or GIP expression cassettes

BIV vectors capable of expressing GLP-1 and/or GIP are constructed as described in WO 01/44458, which is incorporated herein by reference in its entirety. Briefly, the expression cassettes as described above are cloned into a BIV vector plasmid. This plasmid is then co-transfected into a BIV producer cell line along with a plasmid expressing a viral envelope protein (e.g. VSVG envelope) and a BIV packaging construct or constructs (WO 01/44458). The supernatant is then harvested from the producer cells. This supernatant contains the GLP-1 encoding BIV vector, which is then used to transduce the desired cell types. Preferably, the BIV vector supernatant is concentrated after harvest.

Example 8

Generation and production of AAV vectors encoding

GLP-1 and/or GIP expression cassettes

AAV vectors encoding GLP-1 and/or GIP can be generated following standard protocols known to those skilled in the art. Briefly, AAV vectors are constructed by the co-transfection method where a plasmid encoding the AAV vector, composed of the AAV inverted terminal repeat (ITR) sequences and the GLP-1 or GIP expression cassette is cotransfected with an AAV helper plasmid as described previously (Xiao et al., 1996). Subconfluent 293 cells are cotransfected with the AAV plasmid and helper plasmid using calcium phosphate precipitation. Forty-eight hours after transfection, the cells are harvested, lysed by 3 cycles of freeze-thawing and sonicated to release the AAV vector. Following ammonium sulfate precipitation, the virus particles are concentrated twice by cesium chloride density gradient centrifugation.

Example 9

In vivo evaluation of a GLP-1 encoding Gutless adenoviral vector

The GLP-1 encoding gutless adenoviral vectors, AGV-C7-GLP, AGV-CMV-GLP, and AGV-ALB-GLP are evaluated in diabetic mice following systemic vector delivery. This example describes the regulated, AGV-C7-GLP vector, but other vectors of the invention are evaluated in a similar manner.

The ability to regulate GLP-1 expression in a diabetic animal model and in humans is desired to potentially improve the utility of the therapy. The ligand-inducible controlled gene expression (CGE) system has been described (PCT/US02/16946). As our animal model, C57BL/6 mice are fed a high fat diet. These animals become obese and diabetic and display elevated fasting blood glucose levels, elevated insulin levels, and impaired glucose tolerance (Desai et al., 2001). The diabetic mice are treated with 6×10^{10} particles of the AGV-C7-GLP vector and an equal amount (6×10^{10} particles) of a gutless vector encoding a novel transcription factor via tail vein injection. This system is similar to that described in Xu, et al. (2002), except Xu, et al. was expressing endostatin and here, GLP-1 is being expressed. This dose of vector results in efficient transduction of the liver with both vectors (PCT/US02/16946). The small molecule inducing agent, such as tamoxifen, or the tamoxifen derivatives described previously (PCT/US02/16946) are delivered at a dose of 50 ug/mouse

through IP injection, daily, dependent on the length of time and level of GLP-1 expression desired. Animals are monitored for GLP-1 expression in the serum by ELISA (Linco Research Inc., St. Charles, Missouri), and for fasting blood glucose levels, insulin levels, and glucose tolerance. Other parameters are also assessed, including lactate, free fatty acids, triglycerides, and liver enzyme levels. The amount of inducing agent can be varied in response to any of these measured parameters.

This example describes an adenovirus encoding an inducible GLP-1 expression cassette and includes another co-administered gutless adenoviral vector which encodes the necessary transcription factor. In a preferred embodiment, both the inducible GLP-1 expression cassette and the necessary transcription factor are encoded on one gutless adenoviral vector.

Evaluation of the AGV-CMV-GLP and the AGV-ALB-GLP vectors is performed in a similar manner. However, 6×10^{10} particles of the relevant vector are delivered via tail vein injection, and no transcription factor vector or inducer is utilized. The same parameters as described above are evaluated.

The disclosures of all patents, publications (including published patent applications), and database accession numbers referred to in this specification are specifically incorporated herein by reference in their entirety to the same extent as if each such individual patent, publication, and database accession number were specifically and individually indicated to be incorporated in its entirety.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

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What Is Claimed Is:

3. A viral vector comprising:

(a) a polynucleotide sequence encoding GLP1;

(b) a polynucleotide sequence encoding a signal sequence upstream of (a); and

(c) a polyadenylation signal downstream of (a).

4. The vector according to claim 1, further comprising a polynucleotide sequence encoding a proteolytic cleavage site located between said polynucleotide sequence encoding GLP1 and said polynucleotide sequence encoding the signal sequence.

3. The vector according to claim 1 or 2, wherein GLP1 comprises SEQ ID NO:2, 4, 6, 8, or 10.

4. The vector according to claim 1 or 2, wherein said polynucleotide sequence encoding GLP1 comprises SEQ ID NO:1, 3, 5, 7 or 9.

5. The vector according to any of claims 1-4, wherein said signal sequence is an IgK signal sequence.

6. The vector according to claim 5, wherein said IgK signal sequence comprises SEQ ID NO:18.

7. The vector according to claim 6, wherein said polynucleotide encoding the IgK signal sequence comprises SEQ ID NO:17.

8. The vector according to any of claims 1-4, wherein said signal sequence is an exendin signal sequence.

9. The vector according to claim 8 wherein said exendin signal sequence comprises SEQ ID NO:16.

10. The vector according to claim 9, wherein said polynucleotide encoding the exendin signal sequence comprises SEQ ID NO:15.

11. The vector according to any one of the preceding claims, wherein the polyadenylation signal is derived from SV40.

12. The vector according to any one of claims 2-11, wherein said proteolytic cleavage site is cleaved by furin protease.

13. The vector according to any one of the preceding claims, wherein expression of said polynucleotide sequence encoding GLP1 is controlled by a regulatable promoter.

14. The vector according to any of claims 1-13, wherein said vector is an adenoviral vector.

15. The vector according to any of claims 1-13, wherein said vector is a retroviral vector.

16. The vector according to any of claims 1-13, wherein said vector is a lentiviral vector.

17. The vector according to any of claims 1-13, wherein said vector is an adeno associated viral vector.

18. The vector according to any one of the preceding claims, further comprising a polynucleotide encoding GIP.

19. The vector according to claim 18, wherein GIP comprises SEQ ID NO:14.

20. The vector according to claim 19, wherein said polynucleotide sequence encoding GIP comprises SEQ ID NO:13.

21. A mammalian cell comprising the vector according to any one of claims 1-20.

22. A packaging cell comprising the vector according to any one of claims 1-20.

23. A method of making a viral particle comprising culturing the packaging cell of claim 22 under conditions wherein the particle is produced.

24. A method of treating diabetes in a mammal comprising administering a physiologically effective amount of the vector in any one of claims 1-20 to the mammal.

25. The method of claim 24, wherein the mammal is a primate.

26. The method of claim 25, wherein the primate is a human.

27. The method of treating diabetes according to any one of claims 24-26, further comprising administering a DPP-IV inhibitor to said mammal.

28. A viral vector comprising:

(a) a polynucleotide sequence encoding GIP;

(b) a polynucleotide sequence encoding a signal sequence upstream of (a); and

(c) a polyadenylation signal downstream of (a).

29. The vector according to claim 27, further comprising a polynucleotide sequence encoding a proteolytic cleavage site located between said polynucleotide sequence encoding GIP and said polynucleotide sequence encoding the signal sequence.

30. The vector according to claim 28 or 29, wherein GIP comprises SEQ ID NO:14.

31. The vector according to claim 30, wherein said polynucleotide sequence encoding GIP comprises SEQ ID NO:13.

32. The vector according to any of claims 28-31, wherein said signal sequence is an IgK signal sequence.

33. The vector according to claim 32, wherein said IgK signal sequence comprises SEQ ID NO:18.

34. The vector according to claim 33, wherein said polynucleotide encoding the IgK signal sequence comprises SEQ ID NO:17.

35. The vector according to any of claims 28-31, wherein said signal sequence is an exendin signal sequence.

36. The vector according to claim 35, wherein said exendin signal sequence comprises SEQ ID NO:16.

37. The vector according to claim 36, wherein said polynucleotide encoding the exendin signal sequence comprises SEQ ID NO:15.

38. The vector according to any one of claims 28-37, wherein the polyadenylation signal is derived from SV40.

39. The vector according to any one of claims 29-38, wherein said proteolytic cleavage site is cleaved by furin protease.

40. The vector according to any one of claims 28-39, wherein expression of said polynucleotide sequence encoding GIP is controlled by a regulatable promoter.

41. The vector according to any of claims 28-39, wherein said vector is an adenoviral vector.

42. The vector according to any of claims 28-39, wherein said vector is a retroviral vector.

43. The vector according to any of claims 28-39, wherein said vector is a lentiviral vector.

44. The vector according to any of claims 28-39, wherein said vector is an adeno associated viral vector.

45. The vector according to any one of claims 28-44, further comprising a polynucleotide sequence encoding GLP1.

46. A mammalian cell comprising the vector according to any one of claims 28-45.

47. A packaging cell comprising the vector according to any one of claims 28-45.

48. A method of making a viral particle, comprising culturing the packaging cell according to claim 47 under conditions wherein the particle is produced.

49. A method of treating diabetes in a mammal, comprising administering a physiologically effective amount of the vector according to any one of claims 28-45 to the mammal.

50. The method of claim 49, wherein the mammal is a primate.

51. The method of claim 50, wherein the primate is a human.

52. The method of treatment as specified in any one of claim 49-51, further comprising administering a DPP-IV inhibitor to said mammal.

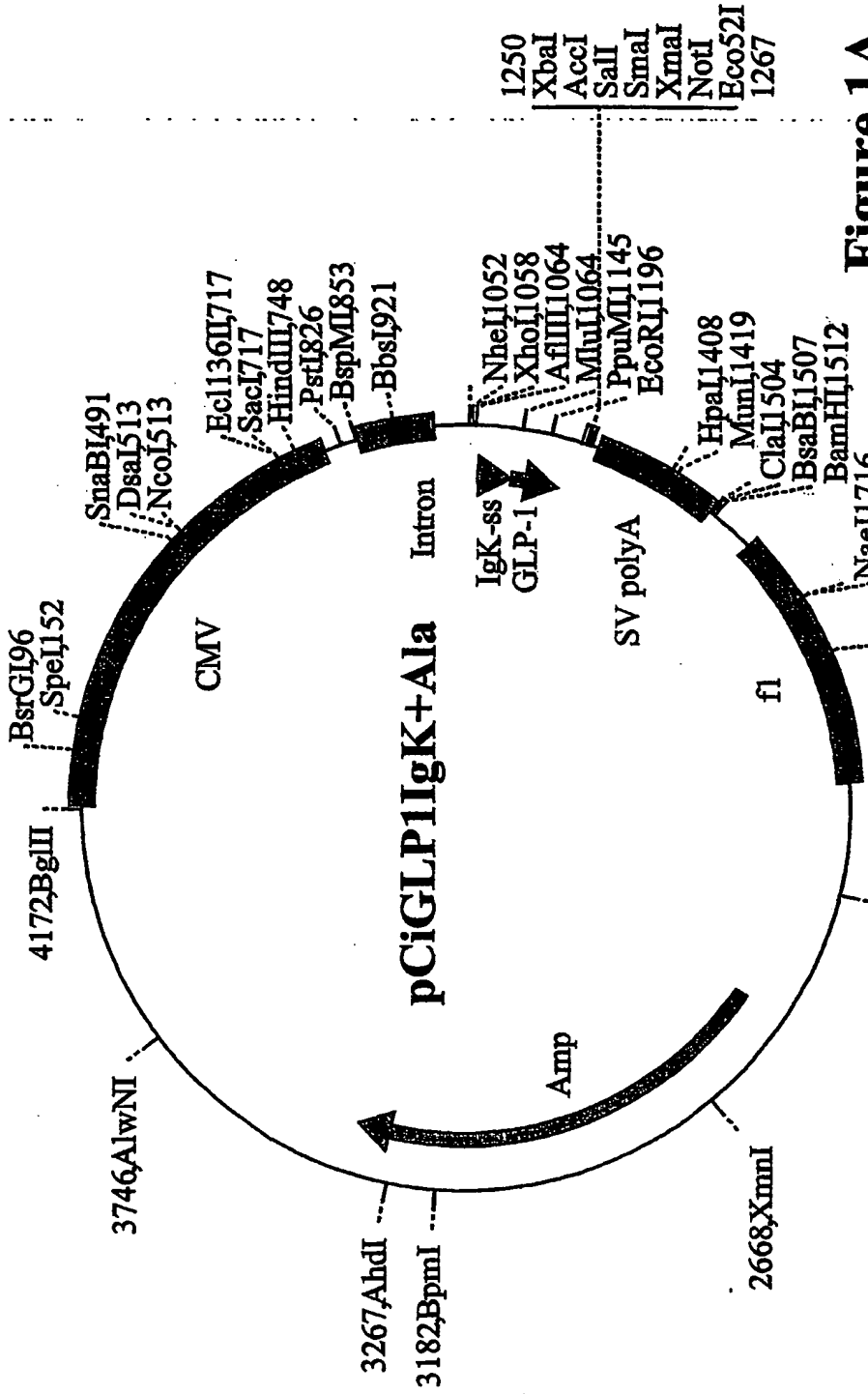

pCiGLP1IgK+Ala
CMV
Intron
IgK-ss
GLP-1
SV polyA
f1
Amp
4172,BglII
BsrGI,96
SpeI,152
3746,AlwNI
3267,AhdI
3182,BpmI
2668,XmnI
2243,NspI
SnaBI,491
DsaI,513
NcoI,513
Ec1136II,717
SacI,717
HindIII,748
PstI,826
BspMI,853
BbsI,921
NheI,1052
XhoI,1058
AflIII,1064
MluI,1064
PpuMI,1145
EcoRI,1196
HpaI,1408
MunI,1419
ClaI,1504
BsaBI,1507
BamHI,1512
NaeI,1716
NgoMIV,1716
DraIII,1819
1250
XbaI
AccI
SalI
SmaI
XmaI
NotI
Eco52I
1267

Figure 1A

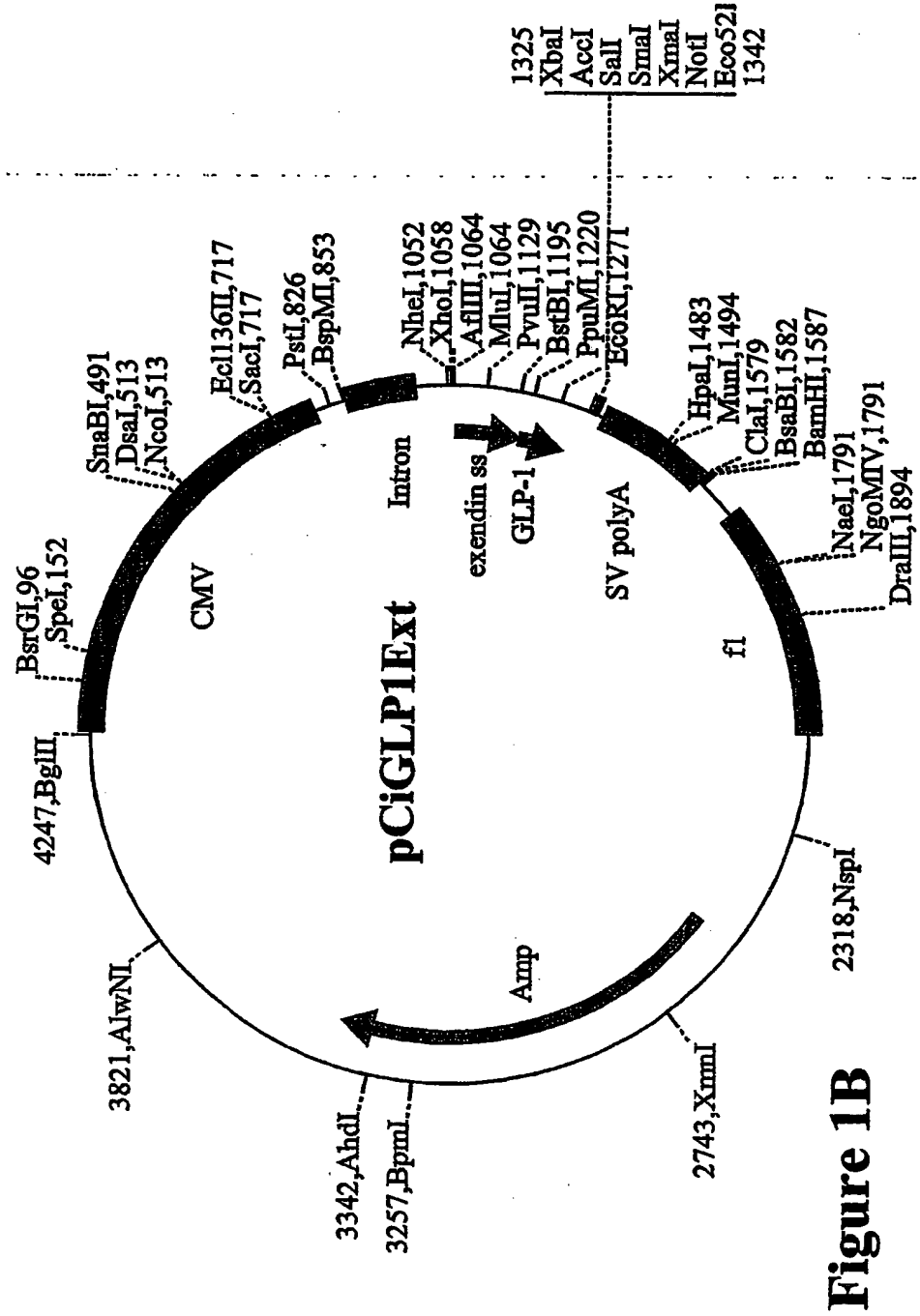

pCiGLP1Ext
CMV
Intron
exendin ss
GLP-1
SV polyA
f1
Amp
BsrGI,96
SpeI,152
4247,BglII
3821,AlwNI
3342,AhdI
3257,BpmI
2743,XmnI
2318,NspI
DraIII,1894
NgoMIV,1791
NaeI,1791
BamHI,1587
BsaBI,1582
ClaI,1579
MunI,1494
HpaI,1483
EcoRI,1271
PpuMI,1220
BstBI,1195
PvuII,1129
MluI,1064
AflIII,1064
XhoI,1058
NheI,1052
BspMI,853
PstI,826
SacI,717
Ecl136II,717
NcoI,513
DsaI,513
SnaBI,491
1325
XbaI
AccI
SalI
SmaI
XmaI
NotI
Eco52I
1342

Figure 1B

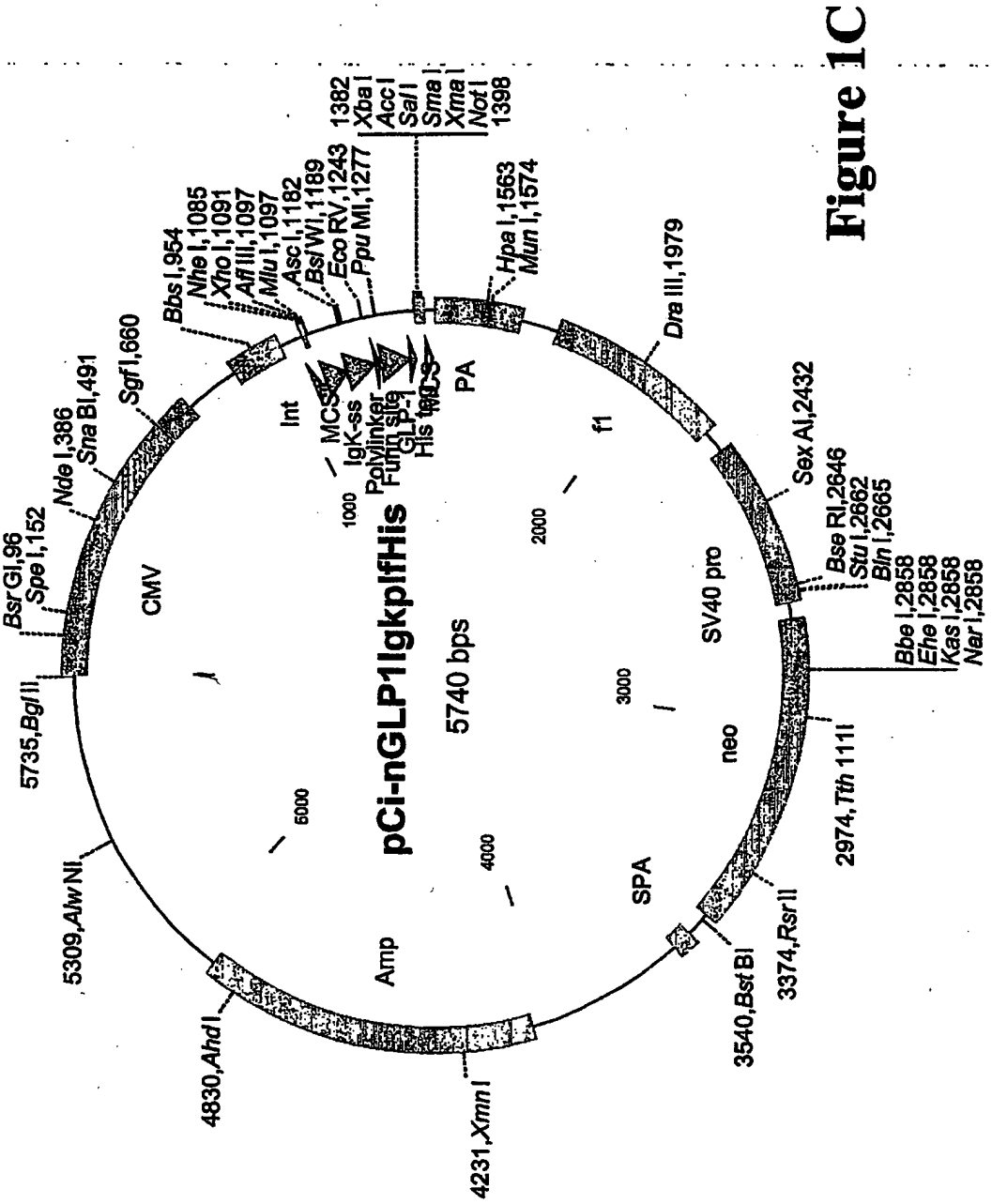
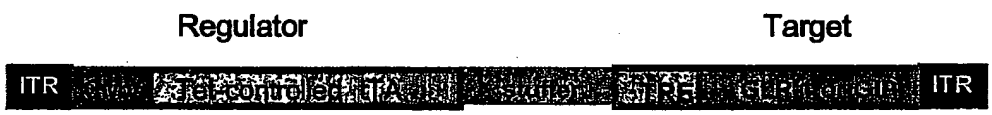

pCi-nGLP1IgkpIfHis
5740 bps
1000
2000
3000
4000
6000
CMV
Int
MCS
IgK-ss
Polylinker
Furin site
GLP-1
His tag
PA
f1
SV40 pro
neo
SPA
Amp
1382 Xba I
Acc I
Sal I
Sma I
Xma I
Not I 1398
Bbs I,954
Nhe I,1085
Xho I,1091
Afl III,1097
Mlu I,1097
Asc I,1182
BsiWI,1189
Eco RV,1243
Ppu MI,1277
Hpa I,1563
Mun I,1574
Dra III,1979
Sex AI,2432
Bse RI,2646
Stu I,2662
Bln I,2665
Bbe I,2858
Ehe I,2858
Kas I,2858
Nar I,2858
2974,Tth 111I
3374,Rsr II
3540,Bst BI
4231,Xmn I
4830,Ahd I
5309,Alw NI
5735,Bgl II
Bsr GI,96
Spe I,152
Nde I,386
Sna BI,491
Sgf I,660

Figure 1C

Adenoviral Vectors EncodingTet System

Gutless Adenoviral Vector encoding Tet-On Regulator



Gutless Adenoviral Vector encoding Tet-Off Regulator

Tetracycline-controlled transactivator (tTA), Tet off (active - tet)

Reverse tetracycline-controlled transactivator (rtTA), Tet on (active + tet)

Tetracycline-response element (TER)

Figure 2

Figure 3A

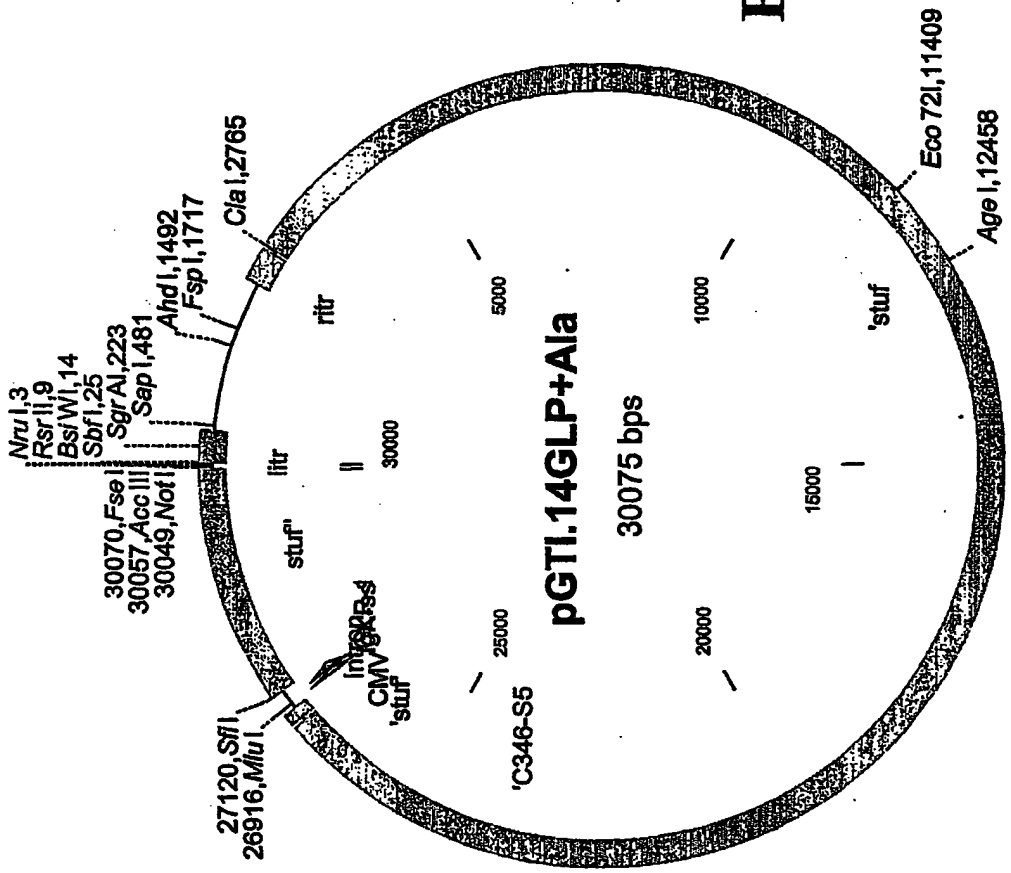
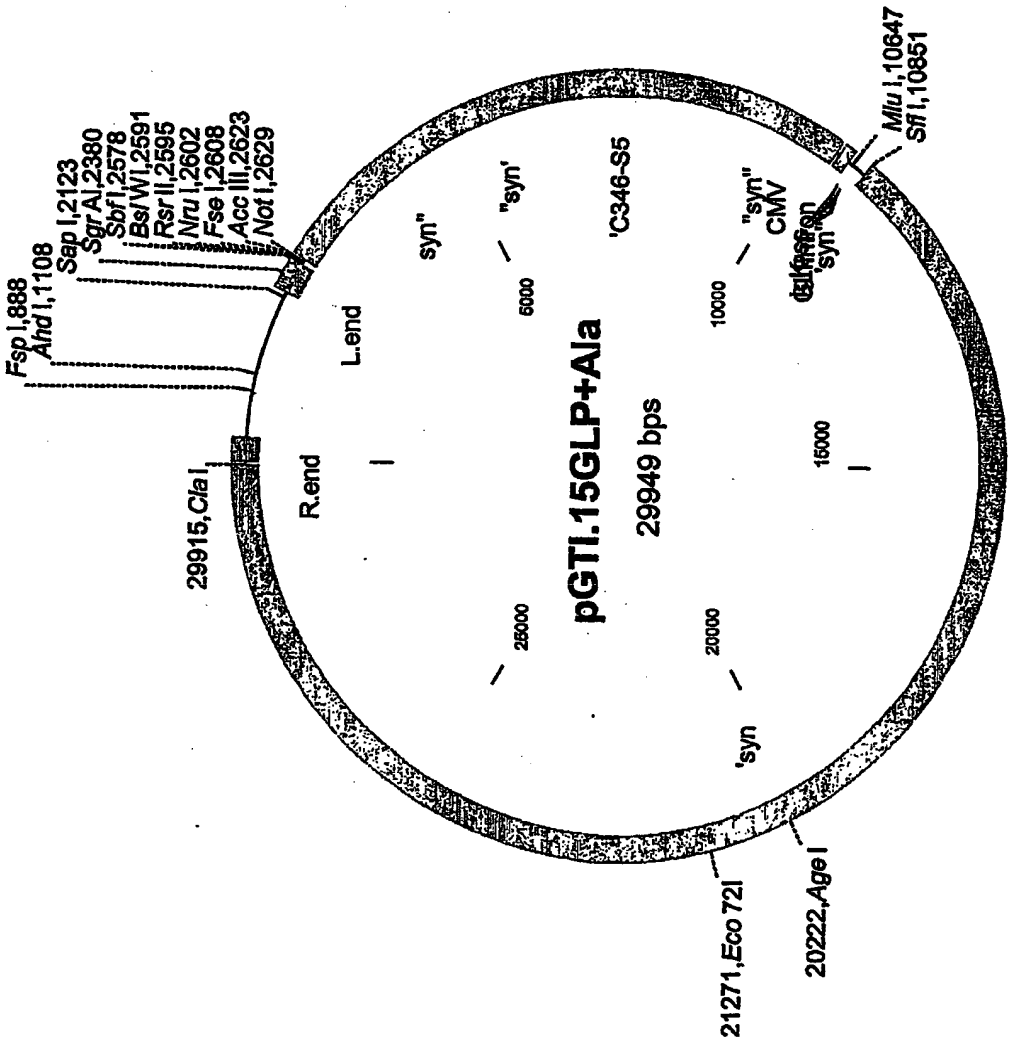

pGTI.14GLP+Ala
30075 bps
5000
10000
15000
20000
25000
30000
'stuf
ritr
litr
stuf'
'C346-S5
'stuf'
CMV
Eco72I,11409
AgeI,12458
ClaI,2765
AhdI,1492
FspI,1717
SgrAI,223
SapI,481
NruI,3
RsrII,9
BsiWI,14
SbfI,25
30070,FseI
30057,AccIII
30049,NotI
27120,SfiI
26916,MluI

Figure 3B


pGTI.15GLP+Ala
29949 bps
R.end
L.end
29915,Cla I
Fsp I,888
Ahd I,1108
Sap I,2123
Sgr AI,2380
Sbf I,2578
BsrW I,2591
Rsr II,2595
Nru I,2602
Fse I,2608
Acc III,2623
Not I,2629
Mlu I,10647
Sfi I,10851
21271,Eco 72I
20222,Age I
'C346-S5
"syn"
'syn'
'syn
5000
10000
15000
20000
25000

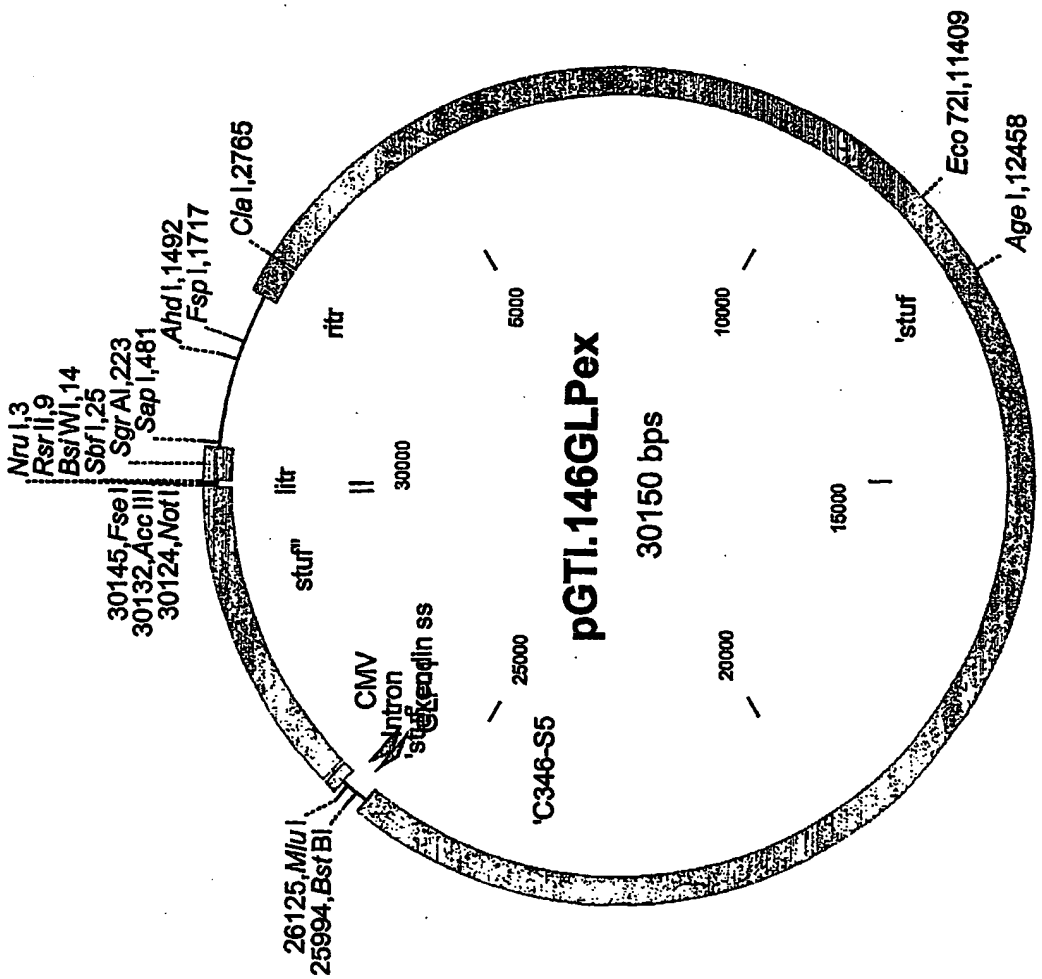
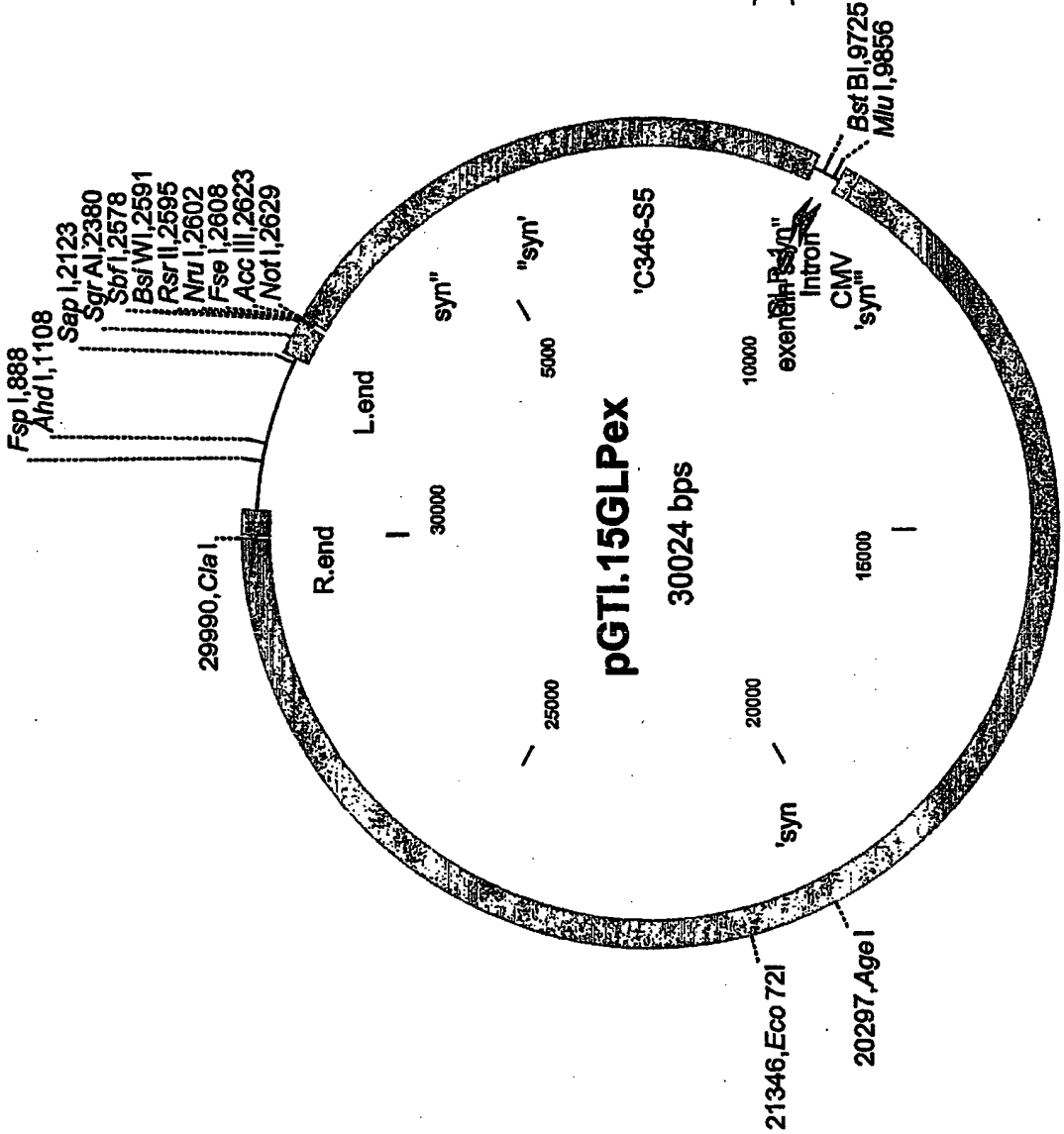

pGTI.146GLPex
30150 bps
5000
10000
15000
20000
25000
30000
'stuf
Eco72I,11409
AgeI,12458
ClaI,2765
AhdI,1492
FspI,1717
SgrAI,223
SapI,481
NruI,3
RsrII,9
BsrWI,14
SbfI,25
30145,FseI
30132,AccIII
30124,NotI
26125,MluI
25994,BstBI
'C346-S5
CMV
Intron
'stuf
ritr
litr
stuf"

Figure 3C

Figure 3D


pGTI.15GLPex
30024 bps
R.end
L.end
'C346-S5
"syn'
syn"
'syn
CMV
'syn'''
Intron
exon1
5000
10000
15000
20000
25000
30000
29990,Cla I
Fsp I,888
Ahd I,1108
Sap I,2123
Sgr AI,2380
Sbf I,2578
BsiWI,2591
Rsr II,2595
Nru I,2602
Fse I,2808
Acc III,2623
Not I,2629
Bst BI,9725
Mlu I,9856
21346,Eco 72I
20297,Age I

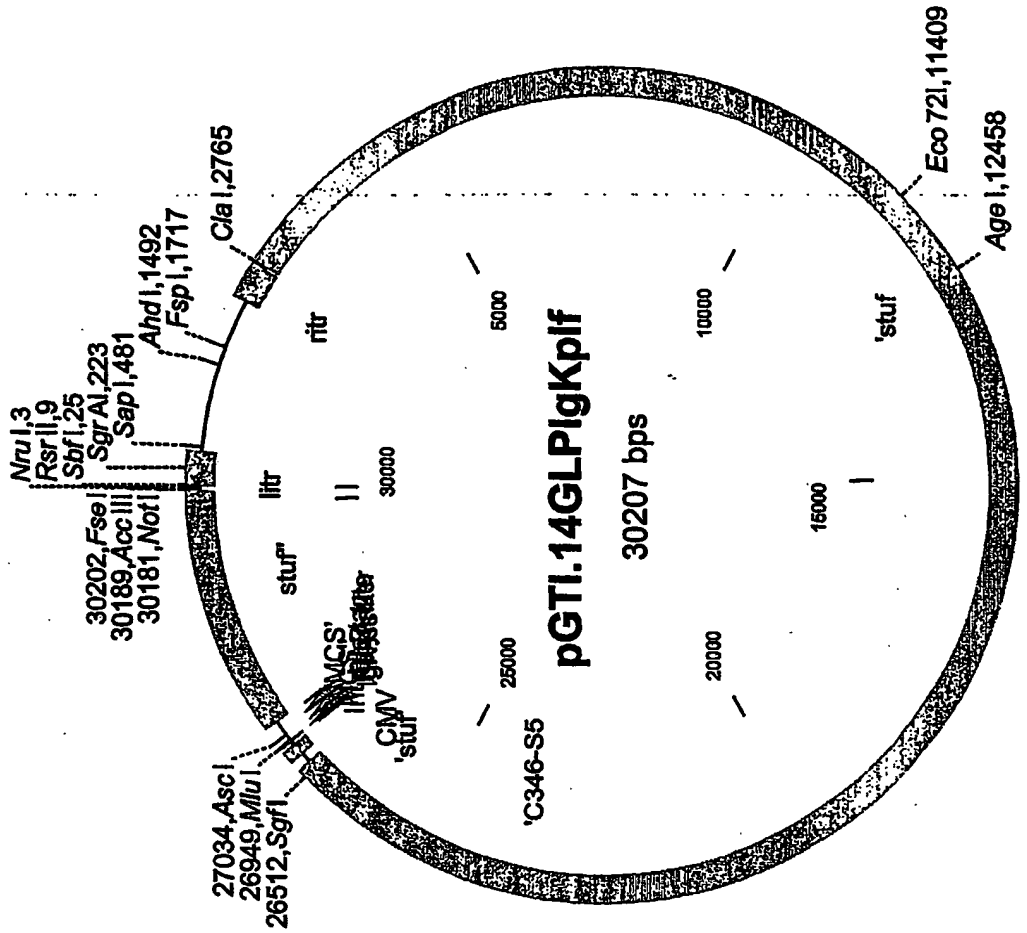

pGTI.14GLPIgKpIf
30207 bps
5000
10000
15000
20000
25000
30000
'stuf
ritr
litr
stuf"
'C346-S5
'stuf
CMV
MGS'
Eco72I,11409
Age I,12458
Cla I,2765
Ahd I,1492
Fsp I,1717
Sap I,481
SgrAI,223
Sbf I,25
Rsr II,9
Nru I,3
30202,Fse I
30189,Acc III
30181,Not I
27034,Asc I
26949,Mlu I
26512,Sgf I

Figure 3E

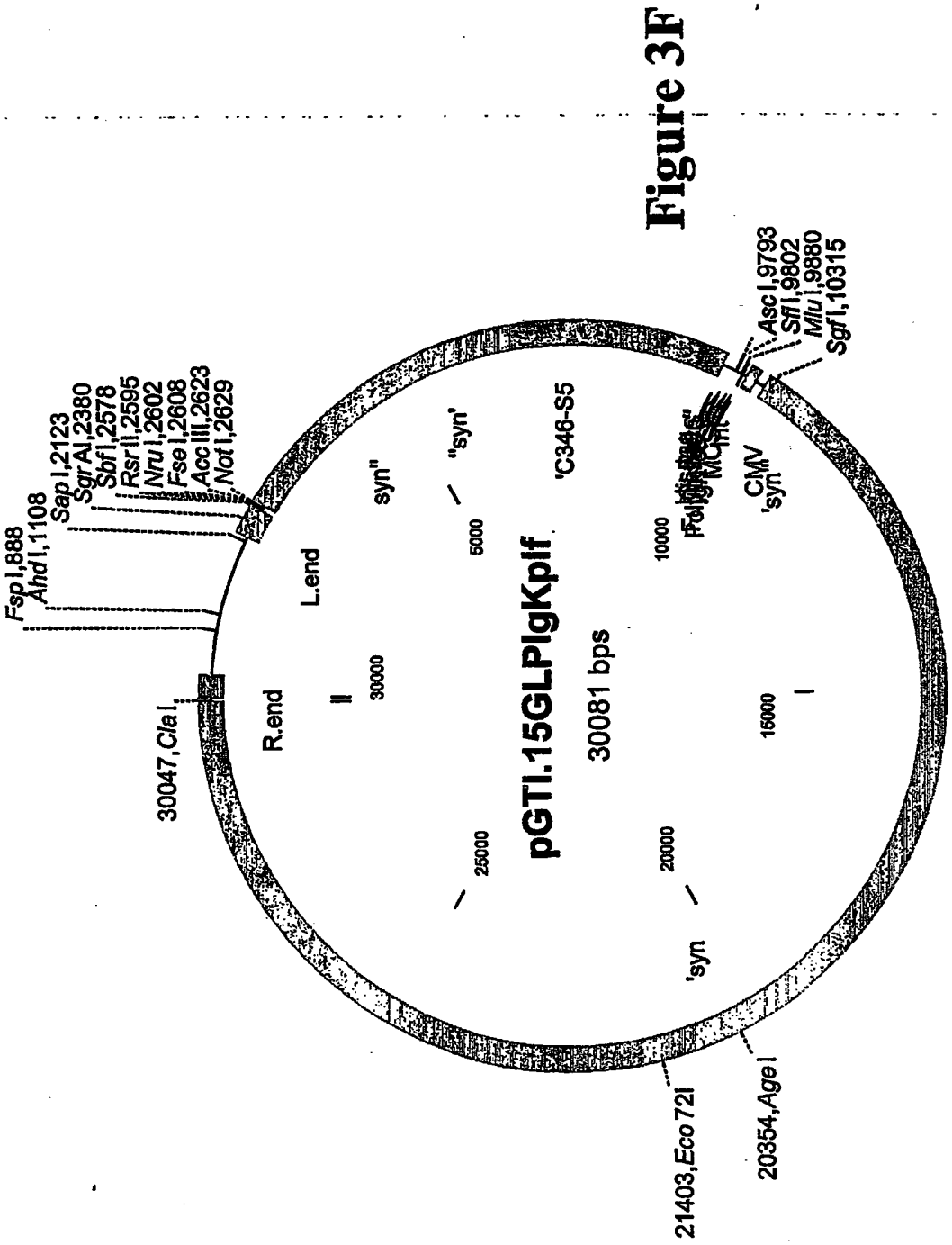

pGTI.15GLPIgKpIf
30081 bps
30047,ClaI
R.end
L.end
syn"
"syn'
'C346-S5
CMV
'syn
'syn
5000
10000
15000
20000
25000
30000
Fsp I,888
Ahd I,1108
Sap I,2123
Sgr AI,2380
Sbf I,2578
Rsr II,2595
Nru I,2602
Fse I,2608
Acc III,2623
Not I,2629
Asc I,9793
Sfi I,9802
Mlu I,9880
Sgr I,10315
21403,Eco72I
20354,AgeI

Figure 3F

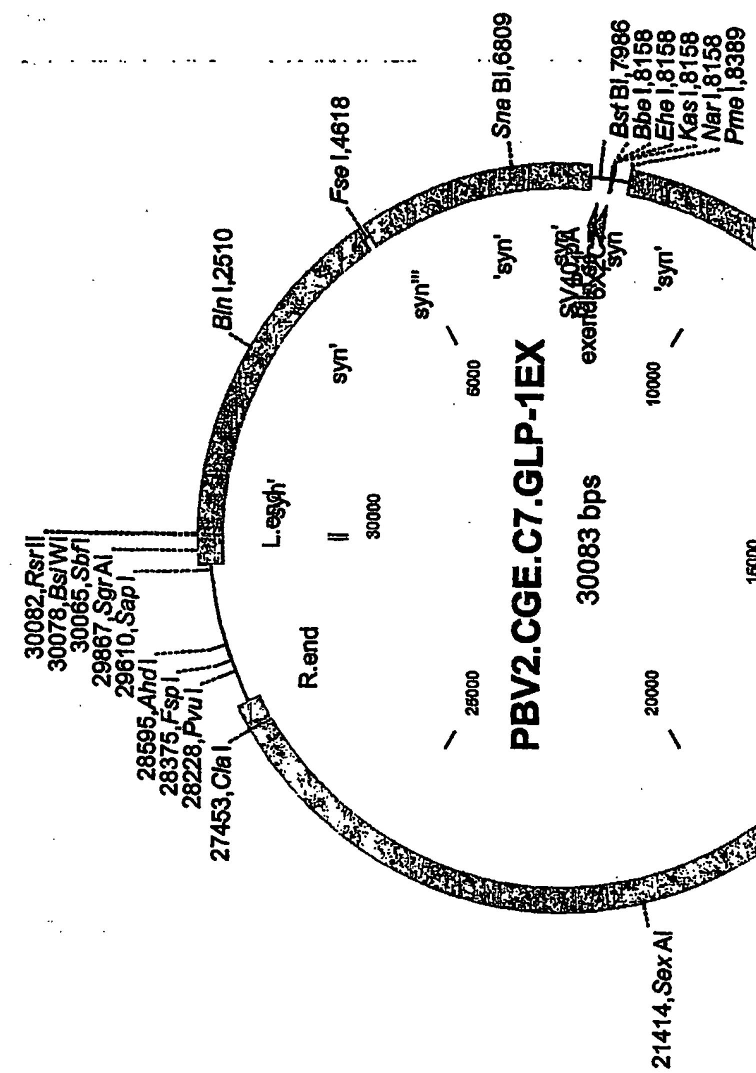

PBV2.CGE.C7.GLP-1EX
30083 bps
30082,RsrII
30078,BsiWI
30065,SbfI
29867,SgrAI
29610,SapI
28595,AhdI
28375,FspI
28228,PvuI
27453,ClaI
21414,SexAI
18809,Eco72I
17760,AgeI
BlnI,2510
FseI,4618
SnaBI,6809
BstBI,7986
BbeI,8158
EheI,8158
KasI,8158
NarI,8158
PmeI,8389
R.end
L.end
syn'
syn'''
'syn'
'syn'
"syn
exend
5000
10000
15000
20000
25000
30000

Figure 4A

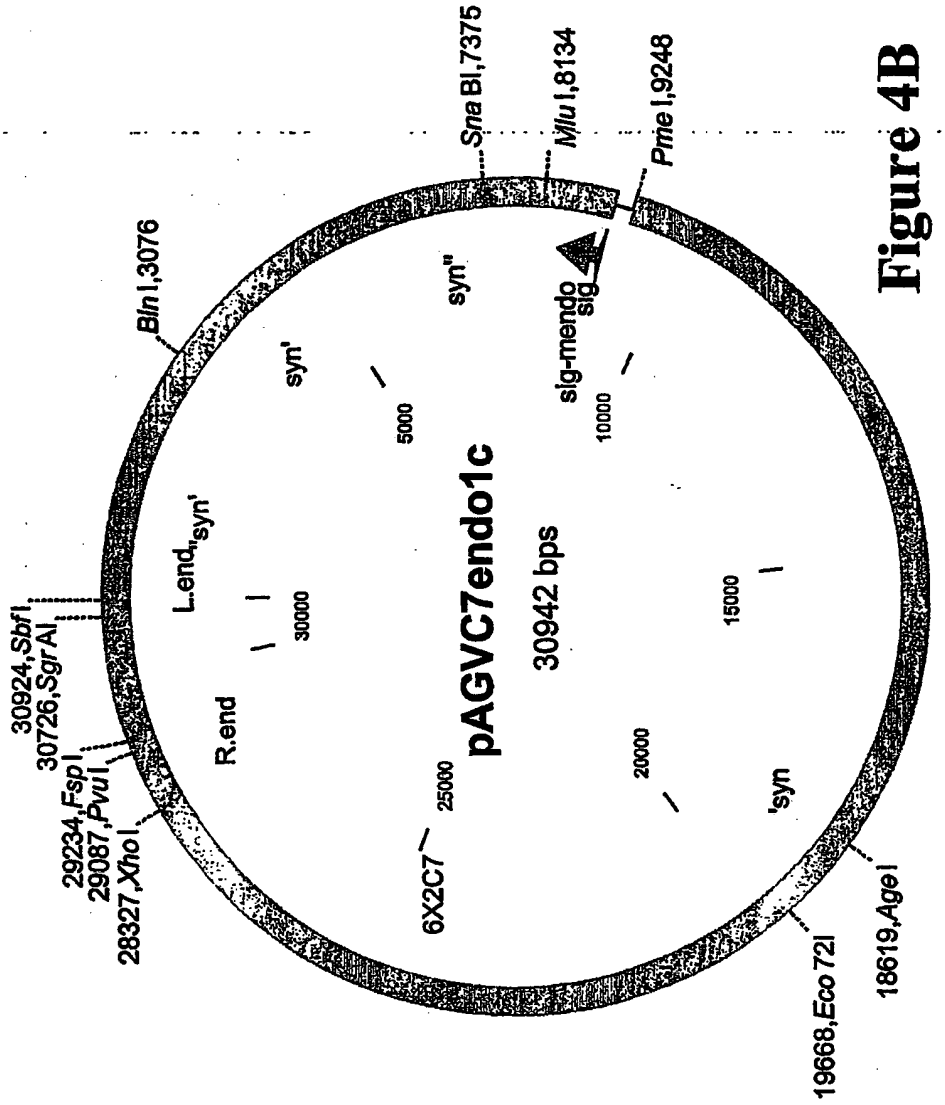

pAGVC7endo1c
30942 bps
Sna BI,7375
Mlu I,8134
Pme I,9248
Bln I,3076
syn'
syn"
sig-mendo sig
L.end"syn'
R.end
6X2C7
'syn
5000
10000
15000
20000
25000
30000
30924,Sbf I
30726,SgrAI
29234,Fsp I
29087,Pvu I
28327,Xho I
19668,Eco 72I
18619,Age I

Figure 4B

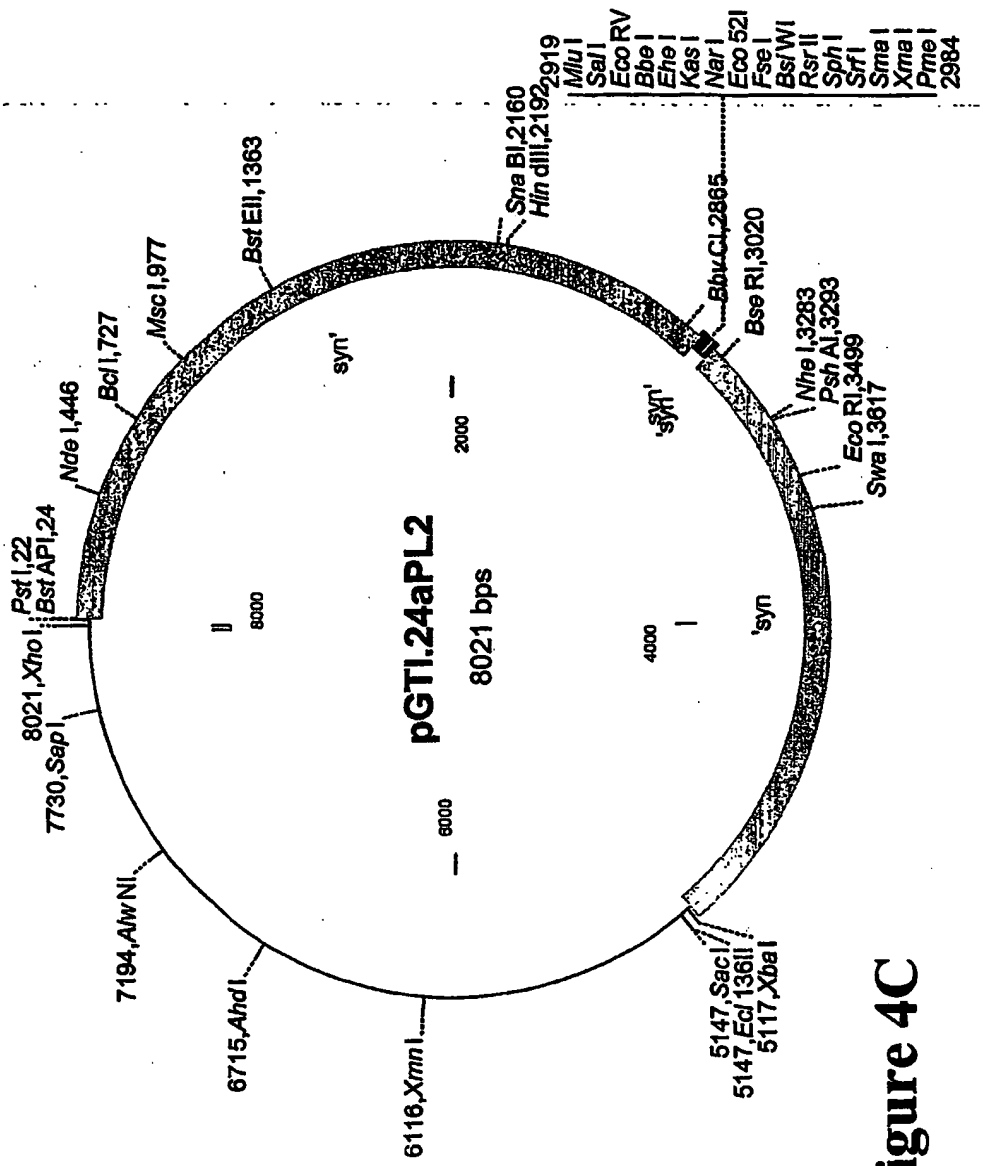

pGTI.24aPL2
8021 bps
2000
4000
6000
8000
syn'
'syn
'syn'
syn
Pst I,22
Bst API,24
8021,Xho I
7730,Sap I
Nde I,446
Bcl I,727
Msc I,977
Bst EII,1363
Sna BI,2160
Hin dIII,2192
2919
Mlu I
Sal I
Eco RV
Bbe I
Ehe I
Kas I
Nar I
Eco 52I
Fse I
Bsi WI
Rsr II
Sph I
Srf I
Sma I
Xma I
Pme I
2984
BbvCI,2865
Bse RI,3020
Nhe I,3283
Psh AI,3283
Eco RI,3499
Swa I,3617
5147,Sac I
5147,Ecl 136II
5117,Xba I
6116,Xmn I
6715,Ahd I
7194,Alw NI

Figure 4C

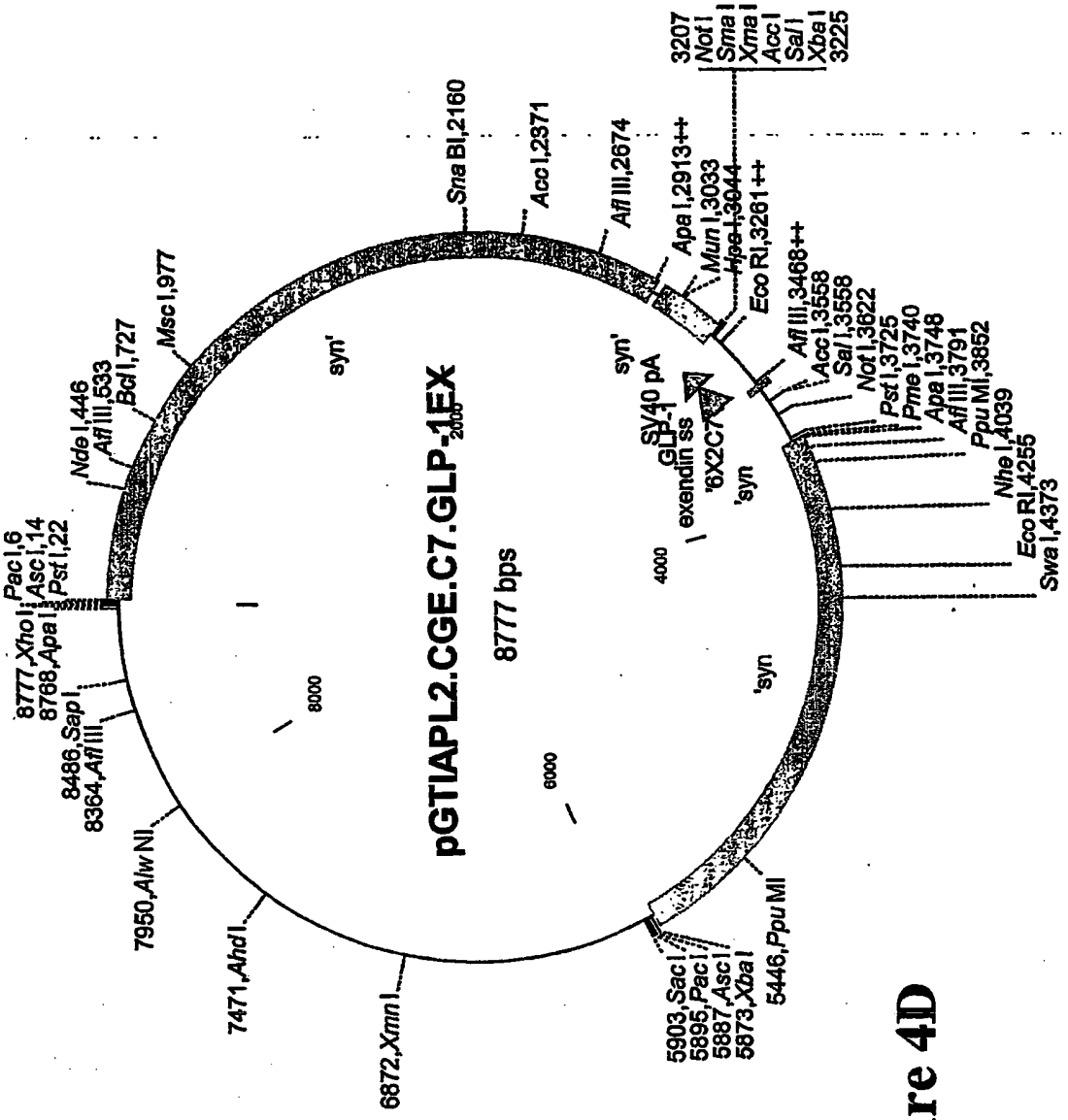

pGTIAPL2.CGE.C7.GLP-1EX
8777 bps
2000
4000
6000
8000
syn'
syn'
'syn
'syn
SV40 pA
GLP-1
exendin ss
'6X2C7
Sna BI,2160
Acc I,2371
Afl III,2674
Apa I,2913++
Mun I,3033
Hpa I,3044
Eco RI,3261++
Afl III,3468++
Acc I,3558
Sal I,3558
Not I,3622
Pst I,3725
Pme I,3740
Apa I,3748
Afl III,3791
Ppu MI,3852
Nhe I,4039
Eco RI,4255
Swa I,4373
3207
Not I
Sma I
Xma I
Acc I
Sal I
Xba I
3225
5446,Ppu MI
5903,Sac I
5895,Pac I
5887,Asc I
5873,Xba I
6872,Xmn I
7471,Ahd I
7950,Alw NI
8364,Afl III
8486,Sap I
8768,Apa I
8777,Xho I
Pac I,6
Asc I,14
Pst I,22
Nde I,446
Afl III,533
Bcl I,727
Msc I,977

Figure 4D

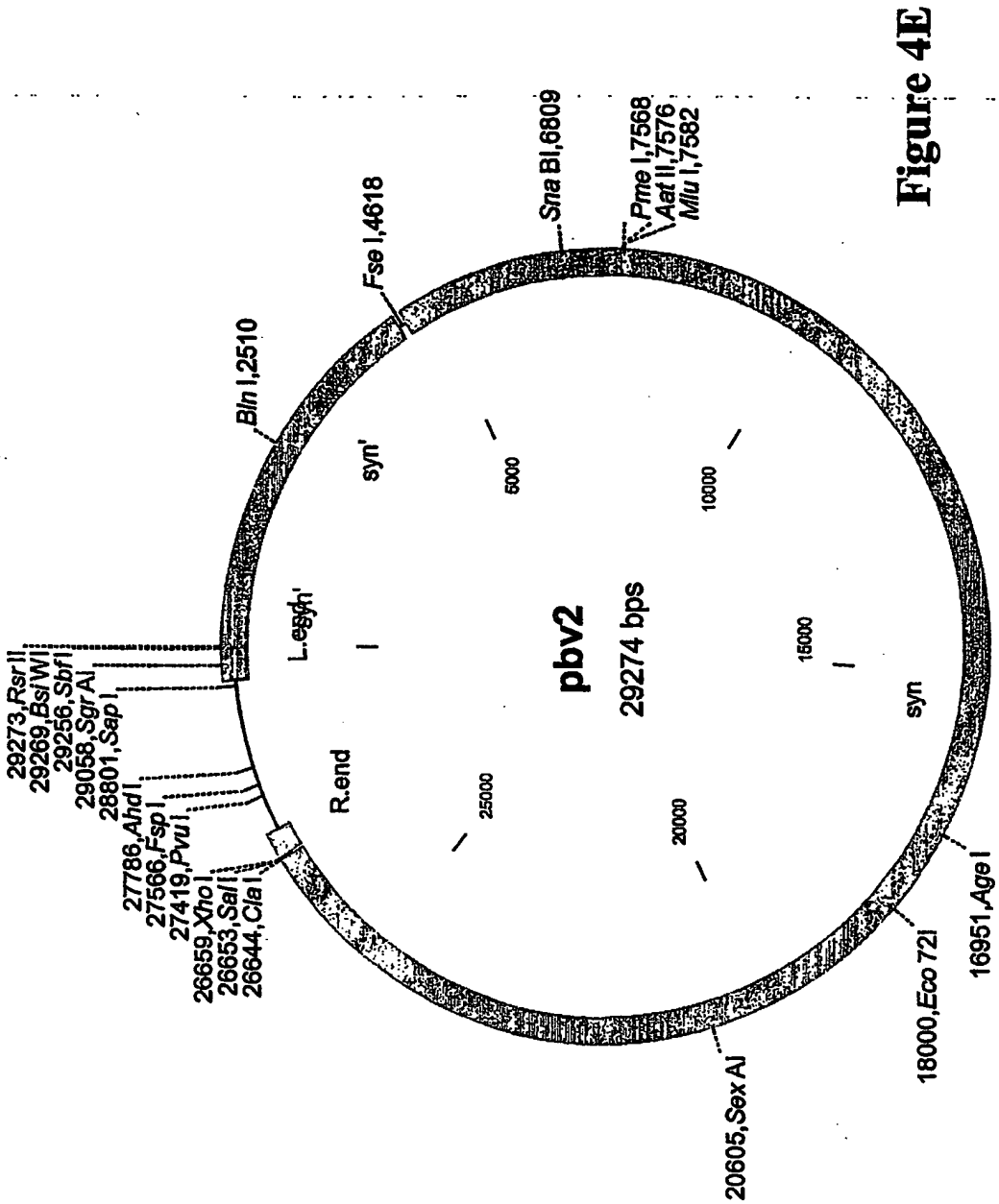

pbv2
29274 bps
5000
10000
15000
20000
25000
L.end
sym'
R.end
syn
Bln I,2510
Fse I,4618
Sna BI,6809
Pme I,7568
Aat II,7576
Mlu I,7582
16951,Age I
18000,Eco 72I
20605,Sex AI
26644,Cla I
26653,Sal I
26659,Xho I
27419,Pvu I
27566,Fsp I
27786,Ahd I
28801,Sap I
29058,Sgr AI
29256,Sbf I
29269,Bsi WI
29273,Rsr II

Figure 4E

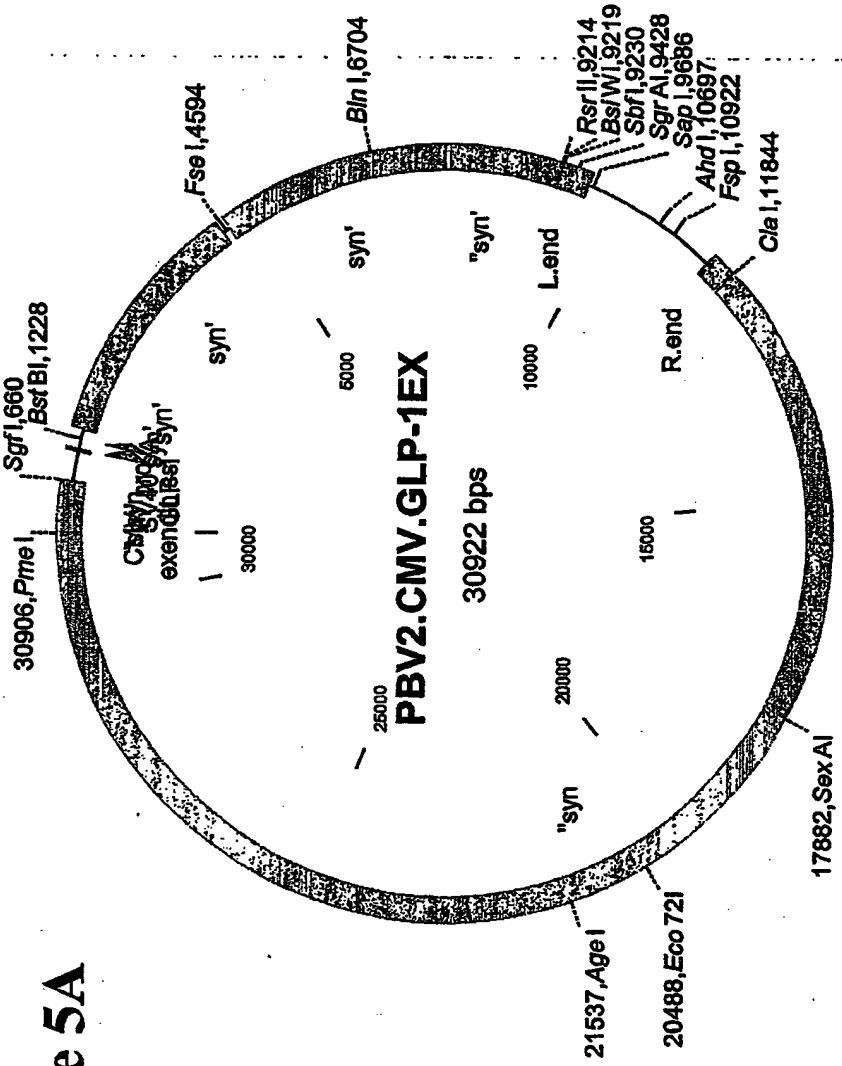

PBV2.CMV.GLP-1EX
30922 bps
Sgf I,660
Bst BI,1228
Fse I,4594
Bln I,6704
Rsr II,9214
BsiW I,9219
Sbf I,9230
Sgr AI,9428
Sap I,9686
Ahd I,10697
Fsp I,10922
Cla I,11844
17882,Sex AI
20488,Eco 72I
21537,Age I
30906,Pme I
syn'
"syn'
L.end
R.end
"syn
5000
10000
15000
20000
25000
30000

Figure 5A

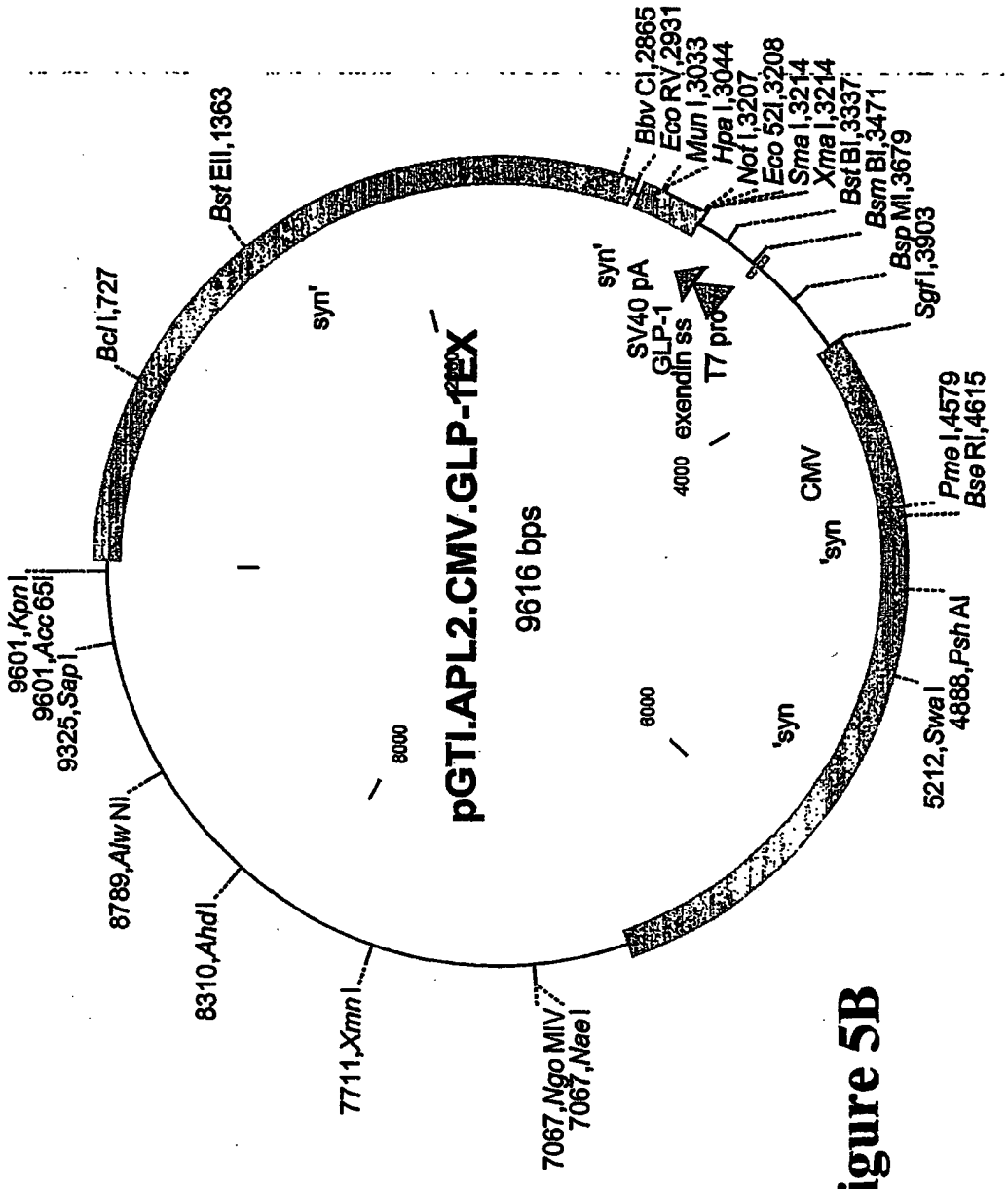

pGTI.APL2.CMV.GLP-1EX
9616 bps
8000
4000
6000
syn'
SV40 pA
GLP-1
exendin ss
T7 pro
CMV
'syn
'syn
Bbv CI,2865
Eco RV,2931
Mun I,3033
Hpa I,3044
Not I,3207
Eco 52I,3208
Sma I,3214
Xma I,3214
Bst BI,3337
Bsm BI,3471
Bsp MI,3679
Sgr I,3903
Pme I,4579
Bse RI,4615
5212,Swa I
4888,Psh AI
9601,Kpn I
9601,Acc 65I
9325,Sap I
8789,Alw NI
8310,Ahd I
7711,Xmn I
7067,Ngo MIV
7067,Nae I
Bcl I,727
Bst EII,1363

Figure 5B

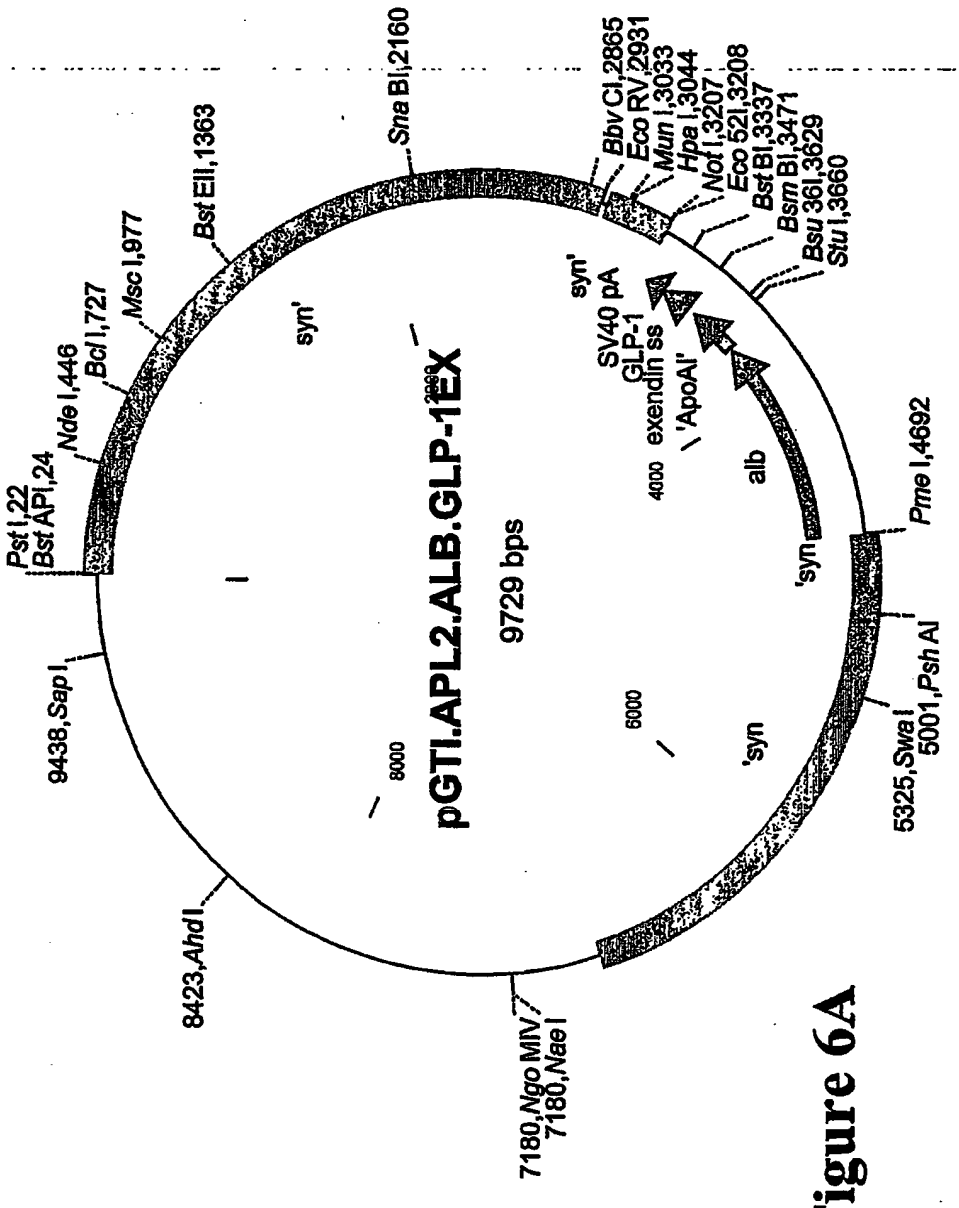

pGTI.APL2.ALB.GLP-1EX
9729 bps
Sna BI,2160
Bbv CI,2865
Eco RV,2931
Mun I,3033
Hpa I,3044
Not I,3207
Eco 52I,3208
Bst BI,3337
Bsm BI,3471
Bsu 36I,3629
Stu I,3660
Pme I,4692
5001,Psh AI
5325,Swa I
7180,Nae I
7180,Ngo MIV
8423,Ahd I
9438,Sap I
Pst I,22
Bst API,24
Nde I,446
Bcl I,727
Msc I,977
Bst EII,1363
syn'
SV40 pA
GLP-1
exendin ss
'ApoAI'
alb
'syn
'syn
'syn
8000
6000
4000

Figure 6A

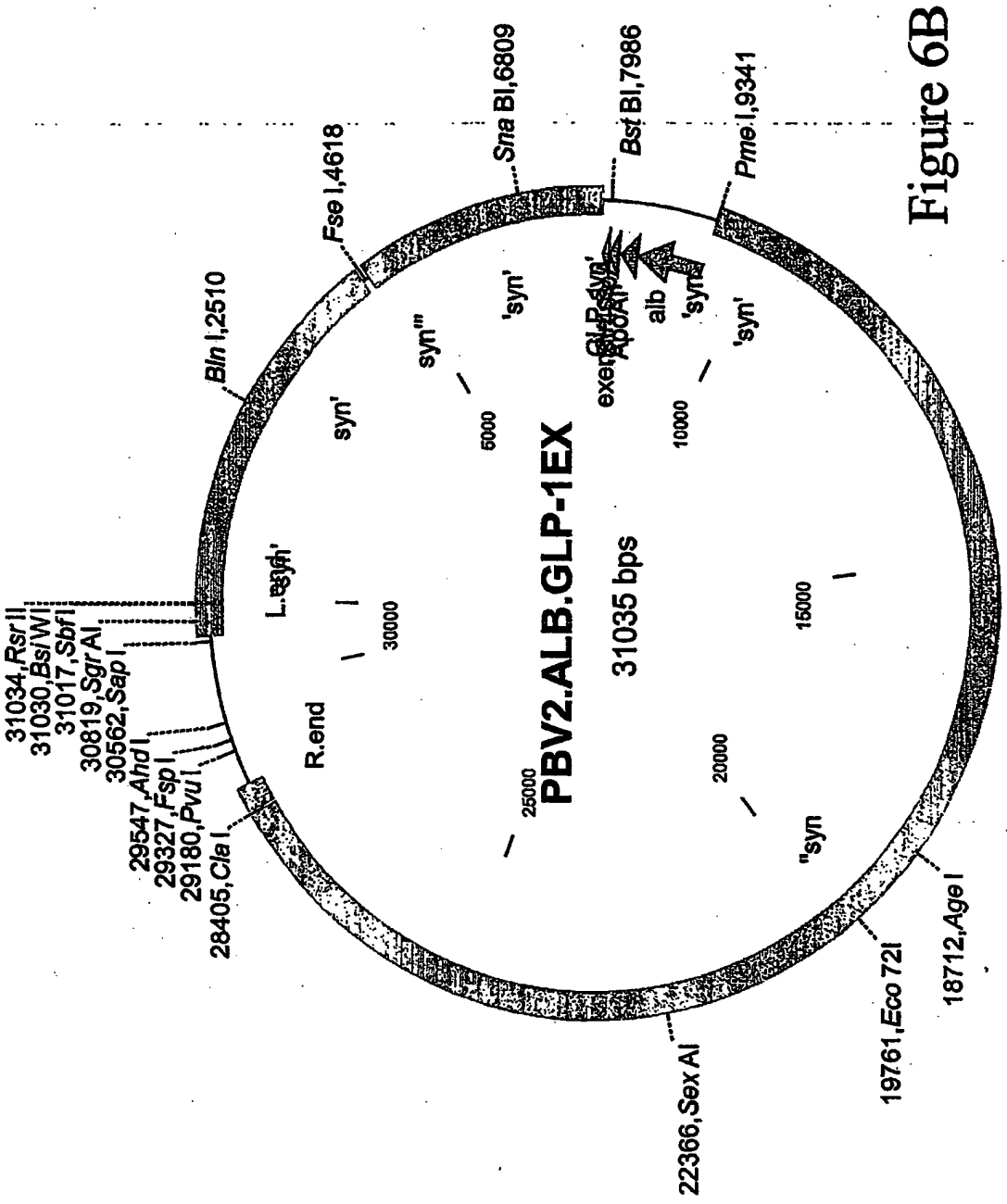

PBV2.ALB.GLP-1EX
31035 bps
5000
10000
15000
20000
25000
30000
Bln I,2510
Fse I,4618
Sna BI,6809
Bst BI,7986
Pme I,9341
Age I
Eco 72I
Sex AI
Cla I
Pvu I
Fsp I
Ahd I
Sap I
Sgr AI
Sbf I
BsiWI
Rsr II
R.end
L.end
syn'
syn''
'syn'
alb
'syn
"syn
18712,Age I
19761,Eco 72I
22366,Sex AI
28405,Cla I
29180,Pvu I
29327,Fsp I
29547,Ahd I
30562,Sap I
30819,Sgr AI
31017,Sbf I
31030,BsiWI
31034,Rsr II

Figure 6B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/32051

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 48/00; C12P 21/00
US CL : 514/12, 44, 866; 435/69.1, 69.5, 252.3, 320.1, 325, 455

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/12, 44, 866; 435/69.1, 320.1, 325, 455

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2002/0065239 A1 (CAPLAN et al) 30 May 2001 (30.05.2001), [0013], [0082]-[0084], [0089], [0090].	1, 3, 4, 14, 15, 17
Y	US 6,110,707 (NEWGARD et al) 29 August 2000 (29.08.2000), column 4, line 13-18, column 6, line 23-54), column 44, line 14-column 47, line 31, column 50, line 5 to column 53, line 50,	1, 11, 13, 14, 15, 17,
Y, P	US 6,444,788 B1 (Staby et al) 03 September 2002 (03.09.2002), column 1, line 49-57, column 20, line 18-49.	1, 3, 4.

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 December 2002 (23.12.2002)	15 JAN 2003
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Maria Marvich, PhD David Fuzo FOR Telephone No. (703) 605-1207

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

PCT/US02/32051

Continuation of B. FIELDS SEARCHED Item 3:
EAST databases USPAT, PGPUB, EPO, JPO, Derwent, IBM-IDN
STN databases Medline, CAPLUS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/32051

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim Nos.: 21-27 and 29-48
because they relate to subject matter not required to be searched by this Authority, namely:
Improper Multiple Dependent

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.